Microfluidic perfusion shows intersarcomere dynamics within single skeletal muscle myofibrils

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The sarcomere is the smallest functional unit of myofibrils in striated muscles. Sarcomeres are connected in series through a network of elastic and structural proteins. During myofibril activation, sarcomeres develop forces that are regulated through complex dynamics among their structures. The mechanisms that regulate intersarcomere dynamics are unclear, which limits our understanding of fundamental muscle features. Such dynamics are associated with the loss in forces caused by mechanical instability encountered in muscle diseases and cardiomyopathy and may underlie potential target treatments for such conditions. In this study, we developed a microfluidic perfusion system to control one sarcomere within a myofibril, while measuring the individual behavior of all sarcomeres. We found that the force from one sarcomere leads to adjustments of adjacent sarcomeres in a mechanism that is dependent on the sarcomere length and the myofibril stiffness. We concluded that the cooperative work of the contractile and the elastic elements within a myofibril rules the intersarcomere dynamics, with important consequences for muscle contraction.

The smallest contractile unit of animal striated muscles is the sarcomere, which is formed from a bipolar array of thick and thin filaments composed mostly of myosin and actin proteins, respectively. The cyclic interaction between myosin and actin driven by ATP hydrolysis drives sarcomere shortening and ultimately, produces force (1, 2). In addition, the sarcomeres are structurally interconnected through the Z disks to form myofibrils (1, 3). Therefore, individual sarcomeres are continuously interacting with each other. The sarcomeres contain titin, an elastic protein that spans the half-sarcomere (4, 5). Titin molecules link all of the different areas of a single sarcomere as well as adjoining sarcomeres, creating an elastic network throughout the length of a myofibril (6, 7). Because the sarcomeres are connected in series in a myofibril, changes in the length of one sarcomere on activation may affect the length of adjacent sarcomeres. Such phenomenon is hereupon referred to as intersarcomere dynamics, which may affect force in ways that are difficult to predict based solely on the sliding filament theory.

The classic force–sarcomere length (FSL) relationship is widely used to predict force from the overlap between thick and thin filaments in a sarcomere during Ca2+ activation, because different sarcomere lengths (SLS) lead to different degrees of filament overlap. However, the presence of intersarcomere dynamics and nonuniformity of SLSs (8) provide additional complexity to the system during myofibril activation. It has been known that the lengths of different sarcomeres change and differ significantly during activation, with consequences for force production. Studies investigating spontaneous oscillatory contractions (9) and the residual force enhancement observed after myofibrils are stretched during activation (10) suggest that changes in individual SLSs are the result of links between the contractile apparatus and intermediate filaments composed mostly of titin molecules. Accordingly, sarcomere nonuniformity during activation may lead to the extension of some sarcomeres with the consequent engagement of titin molecules for passive force production, which enables the myofibrils to stabilize in a given activation condition. Because of technical limitations, the mechanisms governing the interaction of sarcomeres in a myofibril and its consequences for force production remain unclear (11).

In this study, we tested the hypotheses that the mechanical work of one sarcomere effectively communicates with other sarcomeres in series through the passive work of titin and that myofibril mechanics are largely governed by intersarcomere dynamics. To test these hypotheses, we used microfluidic perfusions to locally control one sarcomere or a predetermined group of sarcomeres within an isolated myofibril. In this way, we could manipulate the activation and relaxation of a target sarcomere while measuring the behavior of the other sarcomeres in a myofibril. Our data show that intersarcomere dynamics are regulated through a mechanism that combines the work of the contractile apparatus and intermediate filament systems along myofibrils.

Results

Force Produced by Myofibrils After Point Activation of One Sarcomere.

We tested isolated single sarcomeres and groups of sarcomeres within a rabbit myofibril in three different ranges of initial sarcomere length (SLS), which were chosen so as to induce different initial passive tension: 2.4–2.65 µm (very short; called here “slack”), 2.65–2.9 µm (short), and >2.9 µm (long). A local flow of activation solution, which surrounded ~1 µm of the preparation, resulted in the contraction of one sarcomere, whereas the other sarcomeres in series were maintained in a relaxed state (Fig. 1L and Movies S1 and S2). The striation pattern of the nonactivated sarcomeres remained clear over the repeated activation/relaxation cycles of the

Significance

The sarcomere contains a variety of molecules responsible for force generation in striated muscles. During muscle contraction, many sarcomeres work cooperatively to produce force. The mechanisms behind the interaction among sarcomeres during muscle activation have puzzled scientists for decades. To investigate intersarcomere dynamics, we used microfluidic perfusions to activate a single sarcomere within isolated myofibrils. Using computational sarcomere tracking, we observed that the contraction of one sarcomere affects other sarcomeres in series. Studying the interactions between sarcomeres is crucial, because sarcomere nonuniformity has been long associated with several phenomena in muscle contraction that cannot be easily understood.

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target sarcomeres. The isometric forces produced by isolated single sarcomeres (Fig. 1B) were between ~10 and 60 nN/μm² within the range previously published in the literature (12).

At all SL₁ tested, the targeted sarcomere could overcome the overall myofibrillar passive tension. As long as there was overlap between the thick and thin filaments (SL up to ~3.6 μm), all sarcomeres reached a similar final sarcomere length (SL₂) on activation (Fig. S1B and Movie S3). The force produced by an isolated sarcomere was higher than the force produced by one target sarcomere that was activated within a myofibril (Fig. 1B). Furthermore, when we compared preparations with the same number of sarcomeres in series, the force was higher at longer SLs, suggesting a SL dependence for the force development (Fig. 1B and E). For the same SL₁ group, force transmitted to the precalibrated needles decreased with increasing numbers of sarcomeres in series. These results were reproduced in our mechanical model, in which the elements that compose a myofibril are modeled as either linear or nonlinear spring elements. We initiated the model calculations using the same average SL₁ that we used for the experiments with the myofibrils. When we activated one sarcomere, the force developed by a myofibril decreased nonlinearly as a function of the number of adjacent inactive sarcomeres and was higher at longer SL₂ (Fig. 1C and F). At the end of the contraction, the forces developed in the model were also similar to the experimental forces.

In our system, the measured force is a function of the displacement and stiffness of the microneedles used during mechanical testing. Thus, assuming that the stiffness of sarcomeres within a single myofibril is similar, our data suggest that the measured force is dependent on the magnitude of shortening of the target sarcomere, which is greater at longer SL₁ (Fig. 1D) (P < 0.001). The force sensed by the precalibrated needles results from the active and the passive forces components in the myofibril. Based on the characteristics of titin, it is likely that, at the beginning of activation, individual sarcomere shortening results in an increased strain over the titin molecules of the nonactivated sarcomeres. Because passive force develops in a nonlinear manner (13), contractions starting at longer SL₁ will lead to a greater force development than contractions produced at shorter lengths (for a given change in length). Furthermore, the higher passive tension may cause the activated sarcomere to cease shortening at slightly longer SL₂. Although our data show a lack of statistical difference (Fig. 1C) in the range of SL₂ below 2.00 μm, there is a steep region of the ascending limb of the FSL relation, and thus, even very small differences in SL₂ (that we may have failed to detect) may strongly affect the force. We observed that ≥40 sarcomeres in a myofibril can adjust their length after a point activation, a result that was confirmed with our mechanical model.

**Nonactivated Sarcomeres Adjust Their Lengths After Target Activation of One Sarcomere.** To evaluate (i) how an activated target sarcomere dynamically interacts with adjacent sarcomeres and (ii) how the passive forces in adjacent sarcomeres are adjusted along the myofibril, we measured the SLs (SL₂s; Z disk to Z disk distances) (Fig. S1A) and tracked the A-band displacements of all sarcomeres in response to the activation of a sarcomere in the center of the myofibril (Fig. 2A and B). After activation, the SL₂s were not different among nonactivated sarcomeres (Fig. 2C); all of these sarcomeres responded by slightly stretching (Fig. 2D and Fig. S1) (i.e., there is a mechanical linkage among the sarcomeres). To further investigate the processes leading to these SL adjustments, we tracked the A bands of the myofibrils (Fig. 2B and Fig. S2). The absolute displacement of the A bands was larger in nonactivated sarcomeres located closer to the activation point than in sarcomeres located farther from the activation point. The absolute A-band displacement was dependent on SL₂ and therefore, the initial passive tension of myofibrils (Fig. 2B). When the A-band displacements were normalized, this dependence of SL₂ was not observed (Fig. S3A). These experimental results were well-captured in our mechanical model, which shows a decrease in the displacement of A bands when they are located in sarcomeres away from the activated sarcomere (Fig. S3B).

It has been shown that the elastic network of intermediate filaments can link all force-bearing structures of the sarcomere (14). This mechanism is mainly governed by titin molecules, which are the proteins responsible for passive force development in myofibrils. During our experiments, the local increase in Ca²⁺ leads the target sarcomere to contract and stretch the adjacent sarcomeres, likely enhancing the strain over titin filaments. This strain is passively transmitted across the myofibril via the intermediate filament system, affecting several sarcomeres in series.
During the experiments described above, we noticed a small displacement of the target A band in the activated sarcomere, which may have been caused by half-sarcomere nonuniformity during activation. Moreover, previous studies have suggested that the stabilization of the A band in the center of sarcomeres during muscle contraction is regulated by titin (15, 16). Consequently, we evaluated A-band displacements in sarcomeres by changing the position of the point activation to measure how it affected intersarcomere dynamics. Point activation with the microperfusion system placed at the Z disk led to the activation of two adjoining half-sarcomeres, because two A bands were collapsed together at the end of the activation (Movie S4).

Fig. 3B shows that activation of two half-sarcomeres led to a relatively large displacement from the zero position of at least one of the A bands compared with one target sarcomere activation. However, in this situation, the adjoining sarcomeres displaced less than in conditions where one entire sarcomere was activated. This change in position of the thick filament has been seen before at the beginning of myofibril contraction and is attributed to half-sarcomere mechanics (17), leading us to investigate how the myofibril responds to point activation of just one half-sarcomere. We hypothesized that the asymmetrical behavior results from differential half-sarcomere mechanics. We tested this hypothesis by activating half-sarcomeres using micropipettes with diameters of 0.5 μm to reach a small flow area.

![Fig. 2. Response of sarcomeres after point activation. (A, Upper) A typical view of a myofibril (100x magnification) before, during, and after point activation. For clarity, the side to the right of the activated sarcomere is defined as the positive side, whereas the side to the left of the activated sarcomere is defined as the negative side. Vertical arrows represent the direction of the solution flow (red arrows indicate the large flow; the black arrow indicates the flow delivered via microperfusion). When the microperfusion flow is applied to the target sarcomere, it contracts and produces force. (A, Lower) A-band tracking used for data analysis. Each white circle represents one A band tracked, and each color overlapping the A bands represents the displacement (x, y coordinates for every frame). (Scale bar: 5 μm.) (B) Absolute displacement of A bands in the myofibrils after activation of one sarcomere. The activated sarcomere is represented at the center [slack (n = 31), short (n = 47), and long (n = 67) lengths] of the figure (dotted line). The A bands of the adjacent sarcomeres are displaced toward the activated sarcomere. Activation of the target sarcomere creates a quasymetrical displacement of the nonactivated sarcomeres in the plus and minus sides. (C) SL changes after activation of one sarcomere. Starting from different SLs (circles), the final SLs (diamonds) of the nonactivated sarcomeres do not change substantially after activation. (D) Histogram showing the change in SL (ΔSL) of the nonactivated sarcomeres caused by point activation of one sarcomere. The orange bars represent control measurements that we performed by taking two images from the same myofibril in a relaxed state (~5 s apart). The continuous line shows the Gaussian fit for these control experiments. The dotted line shows the Gaussian fit when three experimental groups are merged into the same analysis. Note that the cure is slightly skewed to the right, suggesting a small stretch of the nonactivated sarcomeres. (E) A-band displacements of myofibrils tested in three conditions: long SL1 (3–3.3 μm; n = 26), long SL1 in rigor solution (n = 26), and very long SL1 (3.35 to ~3.6 μm; n = 22). Error bars represent SEM. Act., activation.

To further test the myofibril adjustment to local activation, we repeated the previous experiments using inflexible needles, so that the total length of the myofibrils would not change significantly as a result of activation, removing partially the effects of end compliance. Myofibrils were tested at long SL1 (2.9–3.3 μm), very long SL1 (3.4–3.6 μm), and long SL1 in rigor solution (i.e., in the absence of ATP and Ca2+), a condition that substantially increases the stiffness of the preparation, because it induces a large number of cross-bridges. For all of these conditions, myofibrils remained able to contract in response to the point activation, suggesting that sarcomeres in series can adapt their lengths in response to a local increase in Ca2+ concentration without significant changes in total myofibril length (Fig. 2E).

There was some shortening observed at each end of the myofibrils as indicated by A-band displacements. However, our mathematical model that incorporates the end compliances was able to predict sarcomere contraction and shortening even with probes of very high stiffness and myofibrils in rigor state (Fig. S3 C and D), strengthening the interpretation that intersarcomere dynamics are not necessarily dependent on changes in total myofibril length.

**Half-Sarcomeres Are Displaced During Activation.** During the experiments described above, we noticed a small displacement of the target A band in the activated sarcomere, which may have been caused by half-sarcomere nonuniformity during activation. Moreover, previous studies have suggested that the stabilization of the A band in the center of sarcomeres during muscle contraction is regulated by titin (15, 16). Consequently, we evaluated A-band displacements in sarcomeres by changing the position of the point activation to measure how it affected intersarcomere dynamics. Point activation with the microperfusion system placed at the Z disk led to the activation of two adjoining half-sarcomeres, because two A bands were collapsed together at the end of the activation (Movie S4).

Fig. 3B shows that activation of two half-sarcomeres led to a relatively large displacement from the zero position of at least one of the A bands compared with one target sarcomere activation. However, in this situation, the adjoining sarcomeres displaced less than in conditions where one entire sarcomere was activated. This change in position of the thick filament has been seen before at the beginning of myofibril contraction and is attributed to half-sarcomere mechanics (17), leading us to investigate how the myofibril responds to point activation of just one half-sarcomere. We hypothesized that the asymmetrical behavior results from differential half-sarcomere mechanics. We tested this hypothesis by activating half-sarcomeres using micropipettes with diameters of 0.5 μm to reach a small flow area.
within the plateau (Eq. 3C). Surprisingly, the side that was displaced more was not always the one adjacent to the activated half-sarcromere. Instead, two behaviors were observed: either (i) the A band did not move (and the Z disk was pulled toward the A band) or (ii) the A band was displaced toward the Z disk (Fig. 3D), explaining the asymmetry observed when two half-sarcomeres are activated together. For simplicity, we will consider one sarcomere that has one half-sarcromere activated. If the A band is displaced from its zero position toward the Z disk of this half-sarcromere, it drags the other half-sarcromere (Fig. 3D, blue). If the A band does not change its position, it pulls the Z disk and the half-sarcomeres (Fig. 3D, orange). In practice, both situations happen in our experiments. Note that these movements do not result in different SL of between the two sides of the myofibril.

**Sarcomeres and Myofibrils May Produce Different Forces.** Sarcomeres comprising a myofibril have a similar structure and cross-section. Thus, if all sarcomeres were able to reach the same SL, they should hypothetically produce similar active forces. To test this hypothesis, we point-activated all sarcomeres within a myofibril (one at a time) and used the average displacement of the ±1 and ±2 A bands in relation to the target sarcomere to estimate the individual sarcomere forces. Although an indirect measurement of the force in this situation, this procedure was based on our observation that the displacement of the A bands surrounding an activated sarcomere is very similar to the displacement of the needles when we activated one sarcomere (mechanically isolated from the myofibril). In general, we found forces varying from 8 to 45 nN/μm² when comparing all sarcomeres tested in different myofibrils (merged data in Fig. 4A), similar to the forces produced by isolated sarcomeres (dotted line in Fig. 4A). We observed a small range of forces among the different sarcomeres within a single myofibril caused by small variations in SL (Fig. 4A). When myofibrils were analyzed individually, it was possible to observe differences in forces produced by sarcomeres in some myofibrils. These differences are likely related to different changes in length caused by nonuniform SLi or SLg.

According to our data, sarcomeres reach similar SL when point-activated, but this activation affects other sarcomeres in series. Therefore, during full myofibril activation, the dynamic work of sarcomeres in series and the differences in SLi among sarcomeres may influence how a myofibril develops force to reach a steady state. In such a state, myofibril internal motion is close to equilibrium. If, during myofibril activation, every sarcomere shortened to the same SL, the force would be a function of the number of sarcomeres in series and the SLi of each sarcomere. We, therefore, tested the hypothesis that a greater number of sarcomeres in series allows a greater shortening magnitude and hence, higher forces. We performed full activation of myofibrils with different numbers of sarcomeres in series at an SLi of ~2.8 μm. We first activated myofibrils with ~25 sarcomeres, and after the first activation, we reduced the number of sarcomeres in series in the preparation by one-half by positioning the holding needle close to the center of the myofibril. We found that myofibrils with a greater number of sarcomeres produced higher forces, regardless of the average SLi (Fig. S4A and D). These results may be attributed to cumulative shortening when more sarcomeres are activated (i.e., a greater shortening of the myofibril may be possible when more sarcomeres are activated) (Fig. S4B and C).

To further test our hypothesis that more sarcomeres in series allow for a greater shortening and to remove the potential confounding effect of myofibril damage during manipulation, we measured the forces produced by the first contraction of 95 myofibrils and compared them with single sarcomeres. We confirmed that myofibrils with more sarcomeres in series shortened more than myofibrils with fewer sarcomeres and produced higher forces (Fig. 4B and D). However, the increase in force in longer myofibrils was not linearly related to the number of sarcomeres in series (Fig. 4B). When the force was normalized by the number of sarcomeres in the myofibrils, they produced less force per sarcomere than single sarcomeres (Fig. 4C).

To evaluate whether the final SL reached during the contractions was in the zone where maximal force is produced, we performed separate experiments to derive an FSL relation for the myofibrils used in this study (Movie S6). We observed an FSL relation similar to that reported previously in other preparations (18), with a plateau between SLi of ~2.1 and 2.7 μm (Fig. 4E). Based on these measurements, we evaluated if the SLi of the myofibrils reached...
optimal filament overlap. In fact, the majority of the myofibrils contracted to the plateau of the FSL relationship or in an area within 70% of the optimum filament overlap (Fig. 4E). There were some sarcomeres in these myofibrils that reached a length longer than those at the plateau of the FSL, likely because of an increased SL nonuniformity (Fig. 4F). Based on these findings, we decided to test the hypothesis that a larger degree of nonuniformity in SLs along the descending limb of the FSL leads to a decrease in the total force produced by the myofibrils.

**Induced Nonuniformity/Deactivation Reduces Total Myofibril Force.**

Over the years, SL nonuniformity has been observed to happen naturally during muscle contraction. To test how nonuniformity of SLs affects the final force reached by the myofibrils, we used the microperfusion system to induce nonuniformity in activated myofibrils. We performed five different sets of experiments. First, we measured the myofibril force when three to five sarcomeres were activated before full myofibril activation (pre-activation group) and compared the results with those of regular full activation (control) (Movie S7). Two segments of the same myofibril were tested for preactivation. The protocol consisted of three cycles of full myofibril activation followed by two cycles of preactivation and a control contraction. When preactivated, the myofibril generated a small increase in force before a larger increase caused by full activation (dashed square in Fig. 5A). Interestingly, we did not observe significant differences in the final forces measured, showing that initial nonuniformities did not affect force development (Fig. 5A). Second, in another series of experiments, nonuniformity was induced either after contraction or before activation by using a low-Ca\(^{2+}\) solution to relax one sarcomere in a fully activated myofibril (Movie S8). We noticed a sudden reduction in the total myofibril force (Fig. 5B) that was reversed after removal of the microperfusion pipette. We then repeated this experiment but used a high-Ca\(^{2+}\) solution with 40 mM 2,3-butanedione monoxime (BDM), which led to a similar reduction in force (Fig. 5C). A reduction in the total myofibril force was also observed by delaying the activation of a small portion of the myofibril (approximately one sarcomere) during a contraction (Fig. 5D). For all of these situations, the target sarcomere was able to contract, although both sides of the myofibril were inducing sarcomere stretching. This finding suggests that one sarcomere is able to pull several activated sarcomeres and produce a force that is close to its maximum level.

Finally, we used a high-strength ionic solution that depletes the preparation from thick myosin filaments (19) (Movie S9). Extraction of the A band results in a sarcomere with only the intermediate filaments that is able to communicate with other sarcomeres through passive forces. However, A-band extraction may also lead to more compliant titin because of the loss of the interaction between titin and myosin filaments. We compared forces developed by the myofibrils before and after the extraction of the A band of one sarcomere. We found a significant reduction in the isometric force compared with experiments in which the A band was intact (Fig. 5E), supporting the idea that the number of sarcomeres is an important factor for total force development. The extraction of one A band had a smaller effect on the total force in myofibrils with a large number of sarcomeres than in myofibrils with a small number of sarcomeres (Fig. 5F). In longer myofibrils, force dropped after the additional extraction of A bands. We performed a few experiments in which we applied a small stretch after extraction of the A band from myofibrils to evaluate if force could be recovered back to the original values. However, the force remained lower than it was before extraction of the A band (Fig. S5 and Movie S10).

**Discussion**

In this study, we assessed intersarcomere dynamics in isolated myofibrils using microfluidic perfusion, a technique that allows the point activation of one (half-) sarcomere within a myofibril while measuring the response of all other sarcomeres. We triggered cross-bridge formation and contraction of target sarcomeres by locally increasing the Ca\(^{2+}\) concentration. We showed that the shortening of an activated sarcomere leads to an internal regulation in the remaining sarcomeres in the myofibril through passive forces. This result shows that intersarcomere dynamics arise from the cooperative work of the contractile and elastic systems, enabling the action of one sarcomere to be transmitted to the others.

We found that single sarcomeres are able to contract as long as there is myofilament overlap (20), reaching similar SLs independent of the SL\(_0\) (with small differences when SL\(_0\) is ~3.6 μm). This behavior is not observed during full myofibril activation (Figs. 1D and 4G), where the SL\(_0\) is highly nonuniform during activation (17). Although passive and active forces have been investigated separately (21, 22), we showed that the passive force component is responsible for the interaction of sarcomeres in series, facilitating the transmission of the force of one sarcomere to others.

Recent studies have shown that titin controls important aspects of thick filament structure, playing an important role in muscle activation (23, 24). In the future, it will be important to consider how the activation of one sarcomere may be sensed by a thick filament from the neighboring sarcomeres. Moreover, it has been suggested that titin can assist muscle contraction at physiological SLs using the stored elastic energy from unfolded
Ig domains (25). The importance of the elastic system was shown in our experiments, in which a few sarcomeres were activated before full myofibril activation (Fig. 5A). There was not a difference in force compared with myofibrils that were not pre-activated, showing that the passive force components hold the nonactivated area of the myofibril at the optimum length for contraction.

Our microfluidic perfusion system allowed the control of a long known phenomenon of muscle contraction, the nonuniform lengths of sarcomeres during activation (26, 27). From the proposal of the sliding filament theory (1, 2), nonuniform behavior of sarcomeres and its effects in force production during contraction have puzzled scientists. We found that, in short myofibrils, enabling the relaxation of one sarcomere either during or before contraction leads to a reduction in the total force (Fig. 5B–D). This result can be explained by the reduction in the total shortening caused by the relaxed sarcomeres. The same reduction was observed when we extracted the A bands from myofibrils (Fig. 5E). However, this reduction in force was attenuated in myofibrils with more sarcomeres in series (Fig. 5F). It is possible that the lack of one sarcomere was compensated for by an increase in shortening of other sarcomeres. Such a mechanism may have important implications, because the structure of the muscle cell may be designed to balance the presence of weaker or damaged sarcomeres along the myofibril.

This study uses a microfluidic perfusion to show the importance of intersarcomere dynamics and the cooperative work of the contractile and elastic proteins in the myofibril, opening possibilities for new venues of investigation in muscle biophysics.

**Methods**

**Experimental Solutions and Experimental System.** Myofibril preparation and imaging were done as described before (28). The protocol was approved by the McGill University Animal Care Committee and complied with the guidelines of the Canadian Council on Animal Care. Rigor solution was 50 mM Tris, 100 mM KCl, 4 mM MgCl$_2$, and 10 mM EGTA, pH 7.0. Relaxing solution was 70 mM KCl, 20 mM imidazole, 5 mM MgCl$_2$, 5 mM ATP, 14.5 mM creatine phosphate, 7 mM EGTA, and pCa$_2^+$ (equal to $-\log([Ca^{2+}])$) 9.0, pH 7.0. Activation solution was 50 mM KCl, 20 mM imidazole, 5 mM MgCl$_2$, 5 mM ATP, 14.5 mM creatine phosphate, 7.2 mM EGTA, and pCa$_2^+$ 4.5, pH 7.0. We used two precalibrated needles to pierce the myofibrils parallel to the sarcomere A bands from myofibrils (Fig. 5E).

The same reduction was observed when we extracted the A bands from myofibrils (Fig. 5E). However, this reduction in force was attenuated in myofibrils with more sarcomeres in series (Fig. 5F). It is possible that the lack of one sarcomere was compensated for by an increase in shortening of other sarcomeres. Such a mechanism may have important implications, because the structure of the muscle cell may be designed to balance the presence of weaker or damaged sarcomeres along the myofibril.

This study uses a microfluidic perfusion to show the importance of intersarcomere dynamics and the cooperative work of the contractile and elastic proteins in the myofibril, opening possibilities for new venues of investigation in muscle biophysics.

**Mechanical Model.** We developed a mechanical model of a half-sarcomere to better interpret our experimental data (SI Text, Figs. 5I–5M, Table S1, and Movie S11) using the framework of Campbell (29).

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