

On the Mechanics of Single Sarcomeres

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1 Introduction

Sarcomeres are the smallest functional contractile units of skeletal and cardiac muscles. Their properties have been associated with corresponding properties on the cell- (fibre) and whole muscle-level. Specifically, the sarcomere force-length relationship, determined first by Gordon et al. (5), is arguably the most basic property associated with sarcomere and muscle function and has been taken as textbook knowledge ever since it was first described. Furthermore, this relationship established the sliding filament (12,15) and the cross-bridge theory (11) on firm ground, as the plateau and descending limb predicted by the theory were precisely reproduced by their experiments (Figure 1). The interpretation given to the descending limb of the sarcomere force-length relationship was that force decreased linearly with decreasing myofilament overlap (or equivalently, increasing sarcomere length) and the associated loss of cross-bridges that could attach to the actin filament.

However, the study by Gordon et al. (5) had severe limitations. Most importantly, sarcomere force and length, the two basic variables of the force-length relationship were not measured. Force was measured at the end of a single isolated fibre, but since steady-state was not reached, fibre forces were approximated by the intersection of two lines fitted to the activation and “creep” curves (slow and continuous increase in force – Figure 2). The force creep was associated with sarcomere length instabilities on the descending limb of the force-length relationship as first sug-

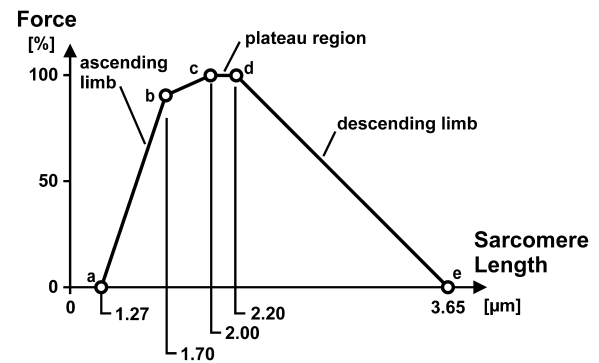


Figure 1: Classic sarcomere force-length relationship for frog skeletal muscle as first described by Gordon et al. (5). Note, the linear decrease of the descending limb of the force-length relationship where loss of force with increasing sarcomere lengths is associated with a decrease in actin-myosin filament overlap and the associated loss of cross-bridge attachment sites.

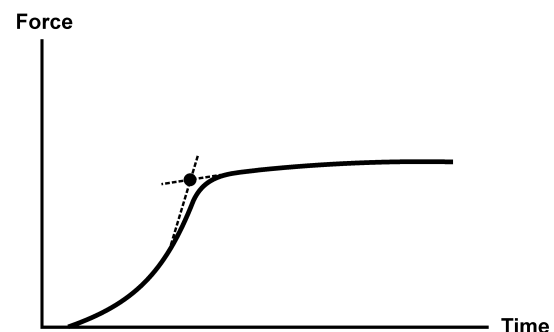


Figure 2: Force-time curve obtained by Gordon et al. (5) and the associated “creep” phase (slow increase in force following full activation). The “dot” indicates the force used by Gordon et al. (5) in their attempt to capture the steady-state force value.

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gested by Hill (10), despite a segment clamp approach used by the investigators.

Similarly, sarcomere length was not measured, but was approximated for the mid-segment that was clamped, by dividing the length of the mid-segment by the estimated number of longitudinally arranged sarcomeres within that segment. Sarcomere lengths in other parts of the fibre were stretched or allowed to shorten depending on the fibre length adjustments that were made to keep the clamped segment at a constant length. Thus, in some of the cross-sections along the fibre, segment lengths changed as force measurements were made, and it appears that slow stretching, rather than a redistribution of sarcomere lengths in the clamped segment of the fibres, was responsible for the force creep.

In summary, Gordon et al. (5) did not measure sarcomere forces, they did not reach a steady-state situation (as required for force-length property assessment), they did not measure sarcomere lengths (or their distribution) within the target segment, and they did not control length changes outside the target segment. Therefore, their results, which were interpreted as representing the force-length properties of single sarcomeres, should have been interpreted with caution and should have been verified independently when appropriate techniques became available.

Recent evidence suggests that the force-length properties of muscles and fibres are not unique but depend on the history of contraction (e.g., 1,4,8). If this was also correct for sarcomeres, one of the most basic assumptions underlying the cross-bridge theory, the idea that steady-state isometric force is uniquely determined by sarcomere length, would need revisiting. Therefore, the purpose of this study was to determine the steady-state isometric forces of sarcomeres in isolated myofibrils. Myofibrils are sub-cellular organelles with single sarcomeres arranged mechanically in series. Therefore, forces measured at the end of myofibrils represent the forces of all and each serially arranged sarcomere, and individual sarcomere lengths can be measured readily. Specifically, we wanted to test if sarcomere length (or equivalently, the amount of actin and myosin overlap) on

the descending limb of the force-length relationship was uniquely related to the isometric, steady-state force as predicted by the cross-bridge theory.

2 Methods

Extraction of single myofibrils and experimental set-up: Strips of rabbit psoas muscle were dissected and tied to small wooden sticks. These samples were stored in a rigor/glycerol (50/50) solution at -20°C. On the day of the experiments, the muscle strips were cut into pieces of about 2mm length using a razor blade, and subsequently blended using previously described protocols (19). The blended muscle was then put into a chamber whose bottom was a glass cover slip placed on top of an inverted microscope (Zeiss, Axiovert 200M, Germany). After sufficient time for stabilization (5-10 minutes), the rigor solution was replaced with a relaxing solution, and myofibrils in suspension were washed away leaving those attached to the bottom of the experimental chamber. A myofibril with a good striation pattern was then selected and attached to a glass needle and motor at one end, and to one nano-lever of a pair (2) which allowed for myofibril force measurements at the other end. Forces were determined by the deflection of the nano-lever to which the myofibril was attached relative to its non-attached reference pair, and the known stiffness of the levers.

The image of the attached myofibril was projected onto a high-density linear photodiode array (Schafter and Kirschhoff Model SK10680DJR, Hamburg, Germany, resolution of 6nm) to give tracings of the myofibrillar striation pattern for identification of the A- and I-bands and the Z-lines. Sarcomere lengths were calculated from Z-line to Z-line, or when these could not be identified reliably, from the centroids of adjacent A-bands.

Protocol: Once a myofibril was ready for mechanical testing, and a clear striation pattern could be observed, a ten-minute rest was given and then the relaxing solution was replaced by the activating solution causing contraction of the myofibril. Six myofibrils were tested isometrically at an average sarcomere length of 2.4 and 3.4 μm and

the active and passive forces were determined. A further twelve myofibrils were activated isometrically at a short length, stretched while activated, and then held isometrically for another 30s until force transients had disappeared. Six of these myofibrils were stretched from a nominal average sarcomere length of 2.4 to 3.4 μm , while the remaining six myofibrils underwent a series of stretches, starting at different average sarcomere lengths and being subjected to variable stretch magnitudes (between 12% and 38% of the initial sarcomere length). For all myofibrils, the mid-diameter was measured using a calibrated eyepiece, and myofibril cross-sectional areas were calculated assuming a cylindrical myofibril shape.

Solutions: The rigor, relaxing and activating solutions were identical to those described previously in our studies (e.g., 19).

Analysis: Isometric, steady-state myofibril forces, and the associated individual sarcomere forces, were determined and plotted against individual sarcomere lengths for the purely isometric contractions, the isometric contractions preceding and the isometric contractions following myofibril stretch. For comparison across myofibrils and sarcomeres, forces were normalized relative to the cross-sectional area of the myofibrils and were expressed as a percentage of the purely isometric reference forces obtained at optimal sarcomere length (2.26-2.43 μm) for rabbit psoas myofibrils (9). Theoretically predicted sarcomere forces were calculated based on the sarcomere force-length relationship published by Gordon et al. (5) for frog but adapted to the myofilament lengths of rabbit psoas (1.08 μm for actin and 1.65 μm for myosin – (6))

3 Results

We confirmed in this study that sarcomeres on the descending limb of the force-length relationship are stable, not only for purely isometric contractions, but also following myofibril stretch onto the descending limb of the force-length relationship (Figure 3). We further confirmed most recent findings that the isometric steady-state forces following myofibril stretch are greater than the forces obtained without prior stretching at that

same length (Figure 4). New to the literature is the finding that individual sarcomere forces following stretch vastly exceed the forces predicted theoretically based on the cross-bridge theory and reported by Gordon et al. (5). Steady-state sarcomere forces following stretch exceeded the purely isometric forces on the plateau of the force-length relationship in eleven of the twelve stretched myofibrils (Figure 5). Across all tests, sarcomere forces following stretch did not follow a trend of decreasing force with decreasing myofilament overlap, rather sarcomere forces of a magnitude equivalent to the purely isometric reference forces at optimal length could be observed for the entire range of the descending limb of the force-length relationship (Figure 5). Similarly, the isometric steady state sarcomere forces at a given sarcomere length could take on a large range of values if they were stretched prior to the isometric contraction.

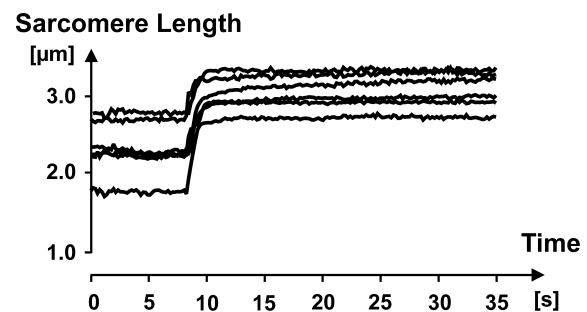


Figure 3: Sarcomere lengths as a function of time for six sarcomeres of one myofibril. Shown are an initial isometric state (flat sarcomere trace), followed by myofibril stretching of about 0.8 μm /sarcomere (increasing sarcomere lengths), followed by a second isometric state (flat sarcomere traces). Note that following stretch all sarcomeres are on the descending limb of the force-length relationship. Note further, that all sarcomere lengths are stable (constant) following stretch, and do not show the instabilities “predicted” in the literature.

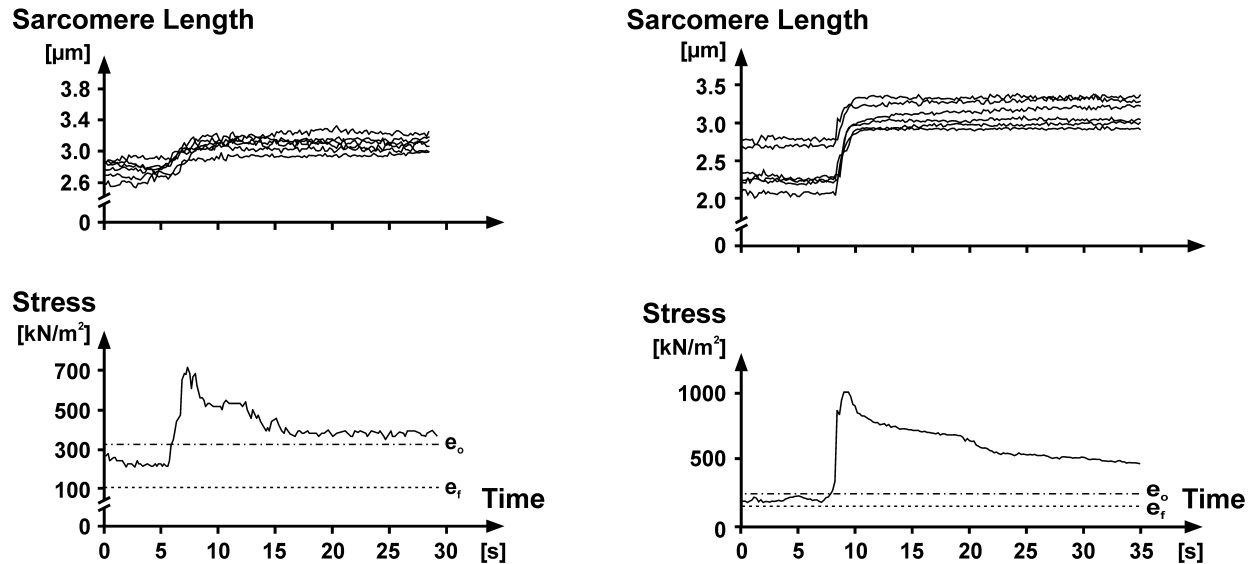


Figure 4: Sarcomere lengths and forces normalized to cross-sectional area (stress) for two representative myofibrils that were stretched onto the descending limb of the force-length relationship. Note that the isometric force prior to stretch is smaller than the isometric force obtained at optimal sarcomere length (e_o – plateau of the force-length relationship). Note further that force following stretch is greater than the isometric force obtained at the final (average) sarcomere length (e_f) and the optimal sarcomere length (e_o).

4 Discussion

The relationship between the steady-state isometric force and length on the sarcomere level was determined in the classic study by Gordon et al. (5). The results of that study agreed perfectly with the theoretical predictions of the sliding filament (12,15) and cross-bridge theories (11,13,14), and are accepted as textbook knowledge. Specifically, the plateau and linear descending limb of the force-length relationship agreed with the idea that isometric steady-state force was uniquely determined by sarcomere length. However, neither sarcomere force nor sarcomere lengths were directly measured in that study, and neither force nor sarcomere lengths along the fibre ever reached steady-state. Therefore, the linear descending limb observed by Gordon et al. (5) does not represent the relationship between an individual sarcomere's length and its associated steady-state isometric force.

Here, we measured for the first time the steady-state isometric forces of sarcomeres and the associated sarcomere lengths in myofibril prepara-

tions. The results indicate that there is a vast region of possible steady-state isometric forces at a given sarcomere length, and that sarcomeres of vastly different lengths can easily co-exist at steady-state on the descending limb of the force-length relationship (Figures 3-5).

Although this study was not aimed at elucidating the mechanisms for the multitude of possible steady-state forces at a given sarcomere length, it is fair to speculate that the contractile history plays an important role in explaining the results. When myofibrils were held isometrically, a curve approximating that predicted by Gordon et al. (5) was obtained, while stretch prior to isometric contractions resulted in increases and shortening (results not shown) resulted in decreases of force compared to isometric. Such phenomena of residual force enhancement following stretching of muscle fibres or whole muscles, and force depression following shortening, have been observed for a long time (e.g., 1,3,4,7,8). However the detailed mechanisms underlying this history dependence of steady-state isometric force remain unclear.

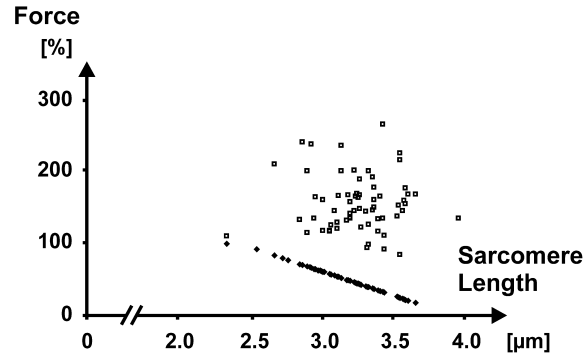


Figure 5: Predicted sarcomere force-length relationship band on the sliding filament theory (filled diamonds – 5) and experimentally obtained isometric steady-state forces of individual sarcomeres following stretches of various magnitudes (open squares). Note that the isometric steady-state forces following stretch are always greater than the corresponding purely isometric forces. Note further, that the isometric forces following sarcomere stretching often exceed the purely isometric forces at optimal sarcomere length (100% force).

However, it has been demonstrated that the structural protein titin, which acts as a molecular spring in muscle tissue and produces much of the passive force in rabbit myofibrils, has a calcium (and therefore activation) dependent regulation of stiffness and so contributes to the increased sarcomere force following stretch (16). However, it has also been ascertained that this “passive” contribution to the increased sarcomere forces is small and that active mechanisms must play an important role (17). Since force enhancement following stretch is not associated with an increase in muscle, fibre or myofibril stiffness, it is safe to assume that the increase in force is associated with an increase in the force/attached cross-bridge. In contrast, the decrease in sarcomere force following myofibril shortening, and force depression in single fibre and muscle preparations, is associated with a concomitant decrease in stiffness (18,20), thereby suggesting that the loss of force following active muscle shortening is caused by a decrease in the proportion of attached cross-bridges, rather

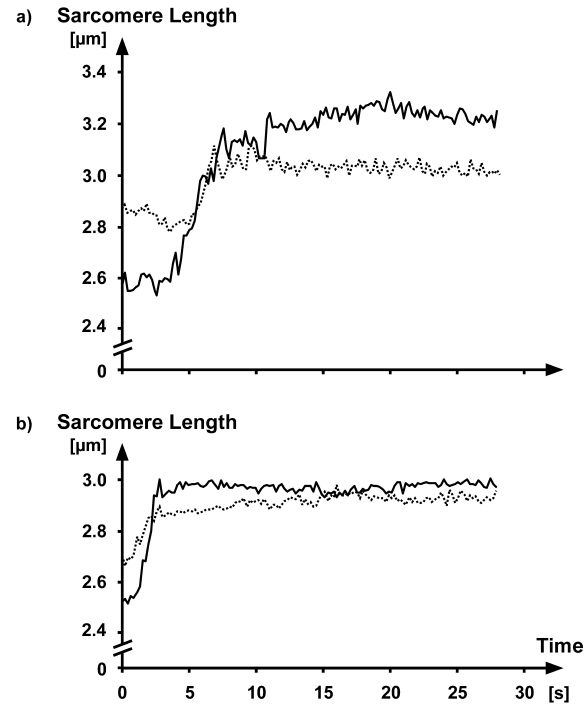


Figure 6: Sarcomere length as a function of time for two sarcomeres each from the same myofibril. The sarcomeres are stretched on the descending limb of the force-length relationship, and while stretched, the initially short sarcomere becomes long. This sarcomere length reversal suggests that passive forces, or a difference in the amount of contractile filament between the two sarcomeres, cannot explain why these two sarcomeres can support the same force.

than a decrease in the force/attached cross-bridge. It might be assumed that the differences in sarcomere lengths in a given myofibril (which must support the same force at any instant in time) might be caused by a difference in the contractile proteins from one sarcomere-to the next, or a difference in the passive forces contributing to the total force of a sarcomere. However, if this was the case, a sarcomere that is short, and another one that is long, would always have to be short and long, respectively, when a myofibril is shortened or stretched on the descending limb of the force-length relationship. However, that is clearly not the case, as illustrated with selected examples

in Figure (6), where sarcomeres switch lengths as they are stretched on the descending limb of the force-length relationship.

In summary, we present first ever steady-state isometric sarcomere forces at a given and controlled level of activation on the descending limb of the force-length relationship where sarcomere force and sarcomere length are measured directly. We found that, in contrast to the predictions of the sliding filament and cross-bridge theory, sarcomere forces are not unique at a given sarcomere length but depend strongly on the contractile history. Although the detailed mechanisms for this sarcomere force enhancement and force depression remain unclear, the idea of a linear descending limb of the sarcomere force-length property, as described in the classic work by Gordon et al. (1966) does not hold. Rather, there is an infinite number of sarcomere force-length relationships that can be obtained by changing the contractile conditions prior to the steady-state isometric force production in sarcomeres. Increasing forces are obtained with increasing stretch magnitudes and decreasing forces are obtained with increasing shortening magnitudes and/or increasing forces during the shortening phase.

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