

Mechanics of Sarcomeres in Series & Instability

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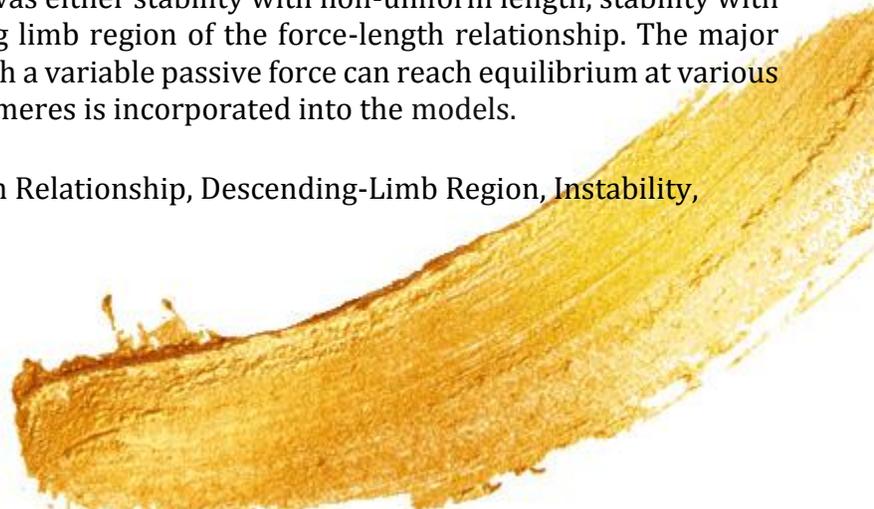
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As a recent graduate of Chemical Engineering with a Biomedical Engineering Specialization at the University of Calgary, Jared Collette worked with the Human Performance Lab in his final years under the supervision of Azim Jinha and Dr. Walter Herzog. His research with the Human Performance Lab centered on computational biomechanics focused on sarcomeres. After exposure to many research topics, biomechanics has been his most passionate subject. He plans to pursue an academic career around biomechanics, starting with a Masters and Ph.D. focusing on developing computational models of cell motility.

Abstract

Sarcomeres are the smallest independent unit of force production in the muscle. Current theoretical models of sarcomere in series, i.e. a myofibril, predict instability on the descending limb region of the force-length relationship. However, experimental evidence suggests that sarcomeres can be stable on the descending limb region with non-uniform lengths. The models presented re-evaluates the assumption that sarcomeres are independent units of contraction. Instead, it is hypothesized that there is a dependency between sarcomeres for force generation. Sarcomeres in series were modelled, with force as the dependent variable and sarcomere length and time as the independent variables. Models were developed with both independent and dependent sarcomere force generation. The independent sarcomere models resulted in instability that current theoretical models predict. Two cases of dependent sarcomere models were implemented, both included a shift in the passive force with varying degrees of dependency between adjacent sarcomeres. With these models, there was either stability with non-uniform length, stability with uniform length, or instability on the descending limb region of the force-length relationship. The major finding was that mathematically, sarcomeres with a variable passive force can reach equilibrium at various lengths if a dependency between adjacent sarcomeres is incorporated into the models.

Keywords: Sarcomere, Myofibril, Force-length Relationship, Descending-Limb Region, Instability, Modelling



Introduction

Muscle Anatomy and Physiology

Sarcomeres are the smallest, independent unit of contraction in skeletal muscles. Within each skeletal muscle cell are long strands of sarcomeres in series called myofibrils, which cause muscle cell contraction. Forces generated by a sarcomere can be broken into active and passive forces. Active forces result from neural stimulation of muscles and are most commonly thought to be generated through cross bridges in accordance with the cross-bridge theory [1]. Passive forces do not involve neural stimulation and are generated from the elasticity contained in sarcomeres, primarily through the protein titin [2]. Early studies have shown that both the active force and the passive force of a single sarcomere depend on the length of a sarcomere, as described by the force-length relationship [3]. The total sarcomere force is the summation of the active and passive forces at any given length.

Myofibril: Characterizing Sarcomeres in Series

The linearized force-length relationship suffices in describing the isometric force of individual sarcomeres when stimulated at a given sarcomere length. However, the application of this relationship to myofibrils leads to instability on the descending limb region. Consider two sarcomeres in series undergoing an isometric contraction initially at two slightly different lengths (Fig. 1). Applying the force-length relationship, the sarcomere with the shorter length will generate a larger contractile force because of the negative force-length slope. With a larger contractile force, this sarcomere will become even shorter and will generate an even larger force. This positive feedback loop is

unstable and would theoretically pull the longer sarcomere to greater lengths until they reach equilibrium with one sarcomere on the ascending limb region (sarcomere length $\approx 2.25 \mu\text{m}$) and the other on the passive force region (sarcomere length $\approx 3.65 \mu\text{m}$). Myofibrils do not experimentally behave this way, and there is a gap between theory and experimental evidence. The models developed in this study are aimed at examining this gap by looking at new ways to model myofibrils.

Literature

Empirical Studies on the Myofibril

One discrepancy discovered in activated myofibrils is the idea of uniform sarcomere length at equilibrium. From the force-length relation, if all sarcomeres are in the same region and have the same force, they must have the same length. However, when stretching single, isolated myofibrils on the descending limb of the force-length relationship, great sarcomere length non-uniformities are observed at equilibrium [4]. When the activated myofibrils in this experiment were stretched, all sarcomeres were rapidly stretched and reached equilibrium at various lengths on the descending limb region of the force-length relationship (Fig. 2). In experiments by Telley and colleagues, the dynamics of cardiac myofibrils were monitored through fluorescent tagging, totaling 100 sarcomeres in series [5]. Of the 100 sarcomeres, 11 were dynamically traced over time. These 11 sarcomeres reached equilibrium at non-uniform length on the descending limb region of the force-length relationship. In that same study, a single myofibril with 80 sarcomeres taken from the soleus muscle of a rat was activated and stretched onto the

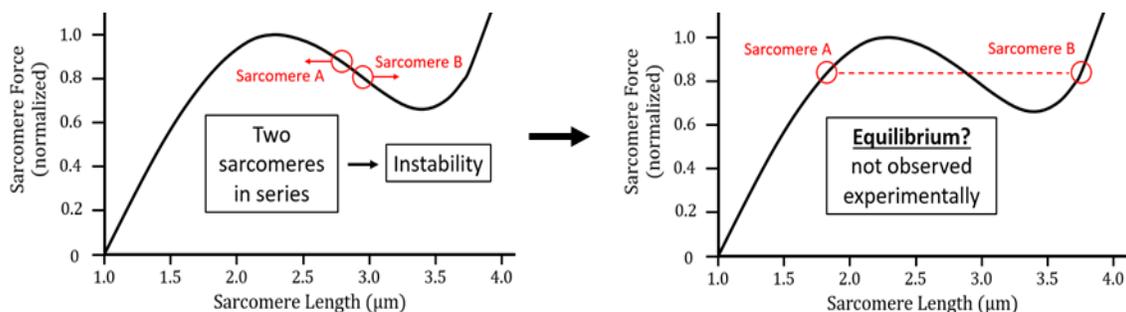


Figure 1: Instability on the force-length curve

descending limb region, and again all sarcomeres reached equilibrium at non-uniform lengths. Other experiments on single myofibril provide similar results as described above [6]–[9].

Theoretical Models on Sarcomeres

To understand the behaviour of sarcomeres, mathematical models have been developed to obtain the force of the sarcomere as a function of length and other factors. A simple empirical model was developed by Gordon et al. in 1966, which is a linearization of the force-length relation (Fig. 3). Passive forces on the force-length relationships can vary greatly between experiments, and those relationships are typically based on approximations of experimentally derived results [10]. However, a first order, linearized relationship can be used to model the passive force.

A frequently used mechanical model of muscle contraction and force production is the Hill’s three element model [11]. Hill’s model uses a contractile element with two non-linear springs, one in series and the other in parallel with the contractile element. The active force, and corresponding force-length relationship, is represented by the contractile element, while the passive force is represented by the parallel spring. More advanced models have been developed for sarcomeres based on springs and dampers, accounting for more intricate anatomical features [12], [13]. For our purposes, a linearized model proved adequate.

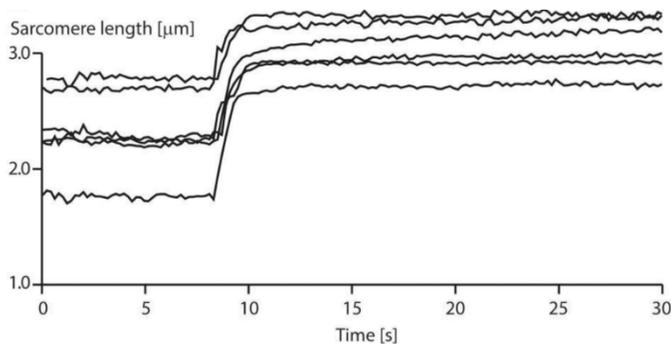
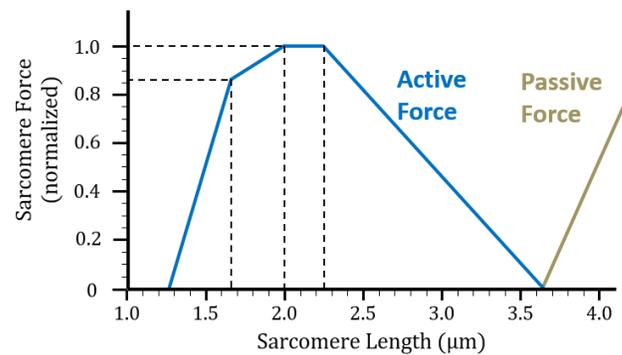


Figure 2: Stability of myofibril on the descending limb region of the force-length curve. Evidence of sarcomere length non-uniformity [4].

Theoretical Models on Myofibrils

Using the simplified, linearized, force-length relationship from Gordon et al. 1966 [3], we developed a theoretical myofibril model of two sarcomeres in series that resulted in instability along the descending limb, contradicting experimental evidence [14]. We then developed two additional models to describe two sarcomeres in series, with the last model incorporating effective stiffness, which tracked experimental data more closely but was limited to two sarcomeres [14].



$$\begin{aligned}
 1.27 < L \leq 1.67 \mu\text{m}, & F^{act} = -2.667 + 2.10 * L \\
 1.67 < L \leq 2.00 \mu\text{m}, & F^{act} = 0.04 + 0.48 * L \\
 2.00 < L \leq 2.25 \mu\text{m}, & F^{act} = 1.00 \\
 2.25 < L \leq 3.65 \mu\text{m}, & F^{act} = 2.592 - 0.71 * L \\
 L \geq 3.65 \mu\text{m}, & F^{pas} = -5.475 + 1.5 * L
 \end{aligned}$$

Figure 3: Linearized force length relationship.

Zahalak, in response to these models, presented his own model of a myofibril using a comprehensive mathematical formulation of stability of sarcomeres in series, which led back to the issue of instability. [15]. Other models that have been developed for myofibrils include a statistical analysis of sarcomeres ignoring individual degrees of freedom that results in sarcomeres converging at non-uniform lengths only on the ascending-limb region instead of the descending-limb region [16]; a model focusing on stored mechanical energy in sarcomeres using contractile units in series and parallel to represent sarcomeres that does not capture the dynamic nature of sarcomeres [17]; and a model focusing on variable compliance of the sarcomere, but focuses on the forces generated by sarcomeres instead of the resulting lengths

produced [18].

Objective and Hypothesis

The purpose of this study was to develop computational models to improve the understanding of myofibril mechanics by modelling sarcomeres in series with non-uniform length. We hypothesized that force generation by sarcomere units in series are not completely independent of each other. This hypothesis was tested by building a dynamic, computational model where sarcomere force is determined by the length of the sarcomere and the two adjacent sarcomeres. Models were developed for sarcomeres independent of each other, simulating myofibrils with two and six sarcomeres in series. Then, models were developed for sarcomeres that were dependent on each other. These last models were developed with 20 sarcomeres in series.

Methods

Overview

The development of the computational myofibril models are broken into three distinct steps. First, all sarcomeres lengths are initialized to a specific region of the force-length curve. Then, the total forces of each sarcomere are obtained based on the sarcomere lengths. This approach is related to the force-length relationship, and it is this step that will vary from model to model. Finally, the length of the sarcomere is updated over a time interval based on the forces produced by each sarcomere. This is repeated until equilibrium is achieved. The variables used for the models are shown in Fig. 4.

Initializing the Models

The sarcomere lengths are initialized to a certain region in the force-length relationship. For the models, a difference of $0.2\mu\text{m}$ was chosen between the minimum and maximum sarcomere lengths. In the models for independent sarcomeres, the sarcomere lengths were initialized randomly within the specified region. In the models for dependent sarcomeres, the sarcomeres lengths are initialized incrementally from the first sarcomere with the minimum

length to the last sarcomere with the maximum length. The difference in lengths between two adjacent sarcomeres remain constant.

Models Developed

To develop models of sarcomeres independent of adjacent sarcomeres, the linearized force length relationship from Gordon et al. (1966) was implemented for a 2 and 6 sarcomere myofibril. Each model was tested on the ascending and the descending limb regions of the force-length relationship.

For models in which sarcomeres were dependent on adjacent sarcomeres, myofibrils containing 20 sarcomeres in series were used. Using 20 sarcomeres allowed the models to discern patterns in sarcomeres groups that were lost with 2-6 sarcomeres, while 20 sarcomeres was still a small enough number to manage individual sarcomeres. The dependence of adjacent sarcomeres on each other was represented using a linear passive force that is shifted to the left or right of the force-length curve, depending on the lengths of the adjacent sarcomeres, described as follows:

$$F^{pas} = b + 1.5 * (L - x_{shift}) \quad (1)$$

$$x_{shift} = f(L_{i-1}, L_{i+1})$$

F = Sarcomere Force

L = Sarcomere Length

x = Shift Left or Right

$b = y - \text{intercept of passive force when } x_{shift} = 0$

$i = i^{th}$ Sarcomere

It is assumed that this shift is linearly proportional to each of the lengths, and the principle of superposition is imposed to combine the terms. Due to the symmetry on either side of a sarcomere, it is also assumed that the constant of proportionality for each length is the same, so the shift can be written as follows:

$$x_{shift} = C_{1,i-1}L_{i-1} + C_{1,i+1}L_{i+1}$$

$$= C_1 * (L_{i-1} + L_{i+1}) \quad (2)$$

To use these equations properly, the parameter 'b' from the passive force equation is

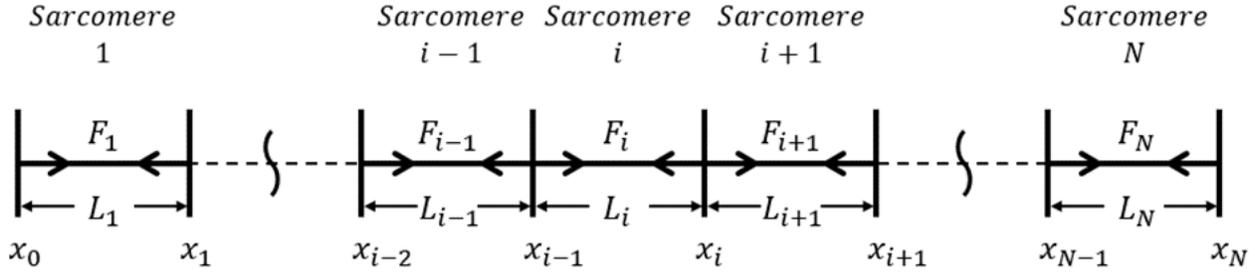


Figure 4: Variable definitions for models. F is the sarcomere force produced from the i th sarcomere, L is the length of the i th sarcomere, and x is the total distance of the i th sarcomere from the start of the myofibril.

required. This 'b' parameter is the y-intercept of the passive force when there is no shift, and is chosen depending on one of two shift methods implemented. In the first method, the constant 'C₁' is a positive value and all shifts occur to the right (i.e. to increasing sarcomere lengths). To choose a b-value, the maximum passive force is chosen for when there is no shift. Based on this curve, the b value is calculated to be $b = -2.475$, leading to the equation

$$F^{pas} = -2.475 + 1.5 * (L - x_{shift}) \quad (3)$$

Three simulations were run to see the effects of 'C₁' on the model. For these simulations C₁ was equal to 0.1, 0.2, 0.3. In the second method, the constant 'C₁' was given a negative value and all shifts occurred to the left (i.e. shorter sarcomere lengths). To choose a b-value, the minimum passive force is chosen for when there is no shift. Based on this curve, the b value is calculated to be $b = -5.475$, leading to the equation

$$F^{pas} = -5.475 + 1.5 * (L - x_{shift}) \quad (4)$$

Three simulations were run to see the effects of 'C₁' on the model. For these simulations, C₁ = -0.15, -0.25, -0.35.

Model Time Evolution

Equilibrium Condition

The following test was used to determine if all sarcomeres were at equilibrium. The average force of all sarcomeres was calculated and was used as a reference value. All forces were compared with the average force and an equilibrium tolerance was set to be ± 0.005 units

normalized to the maximum active force of 1.0 units. If all forces were within this equilibrium tolerance, then the sarcomeres were assumed to be in equilibrium, otherwise they were not accepted to be in equilibrium.

Sarcomere Length Update

Assuming pseudo steady-state motion (i.e. initial velocity and acceleration are zero at each time interval), a linearized function of the change in length can be derived from Newton's second law.

$$\Delta L_{i,t} = C_2 * [F_{i+1,t} + F_{i-1,t} - 2F_{i,t}] \quad (5)$$

Where C₂ is an arbitrary constant that determines the speed of convergence. The force term in the above formula matched the simplest numerical definition of a Laplacian.

Boundary Conditions

For sarcomeres in series, the boundary conditions need to be explicitly defined. Here, the two end sarcomeres i.e. L_1 and L_N , were updated based on the boundary conditions of the sarcomere. For isometric contractions, the total length does not change, and can be represented by the following:

$$\frac{dx_0}{dt} = 0; \frac{dx_N}{dt} = 0 \quad (6)$$

Isometric contractions were assumed for all cases. However, a similar method could be implemented for concentric and eccentric contractions. Applying isometric boundary conditions means that sarcomere lengths are updated as follows:

$$\begin{aligned} L_{1,t+\Delta t} &= L_{1,t} + C_2 * (F_2 - F_1); L_{N,t+\Delta t} \\ &= L_{N,t} + C_2 * (F_{N-1} - F_N) \end{aligned} \quad (7)$$

Results

The results are based on tracking the sarcomere lengths and associated forces over the course of the simulation. For this discussion, stability is defined as sarcomeres that converge to uniform or non-uniform lengths within the descending limb region of the force length relationship, while instability is defined as systems that are not stable.

Sarcomeres Independent of Adjacent Sarcomeres

For the model with two independent sarcomeres in series, initializing the sarcomere lengths in the ascending limb region (1.6-1.8 μm) resulted in stability with uniform sarcomere length. Equilibrium for both sarcomeres was reached at the average initial sarcomere lengths of 1.70 μm and a normalized force of 0.86 (Fig. 5a and Fig. 5b). When initialized on the descending limb region (sarcomere lengths of 2.6-2.8 μm), instability occurred and sarcomere lengths were non-uniform. Equilibrium for the sarcomeres was reached at lengths at 1.52 μm and 3.88 μm and a normalized force of 0.53 (Fig. 5c and Fig. 5d).

For the model with six independent sarcomeres in series, initializing the sarcomere lengths on the ascending limb region (1.6-1.8 μm) resulted in stability with uniform length. Equilibrium for all sarcomeres was reached at a length of 1.68 μm and a normalized force of 0.85 (Fig. 6a and Fig. 6b). When initialized the myofibril on the descending limb region (2.6-2.8 μm), it resulted in instability with non-uniform length. Equilibrium for the sarcomeres was reached with lengths at either 1.52 μm or 3.88 μm at a normalized force of 0.52 (Fig. 6c and Fig. 6d).

Sarcomeres Dependent of Adjacent Sarcomeres

Passive Force Shifted Towards Longer Sarcomere Lengths

With twenty dependent sarcomeres in series, setting C_1 to 0.1 and the initial sarcomere lengths

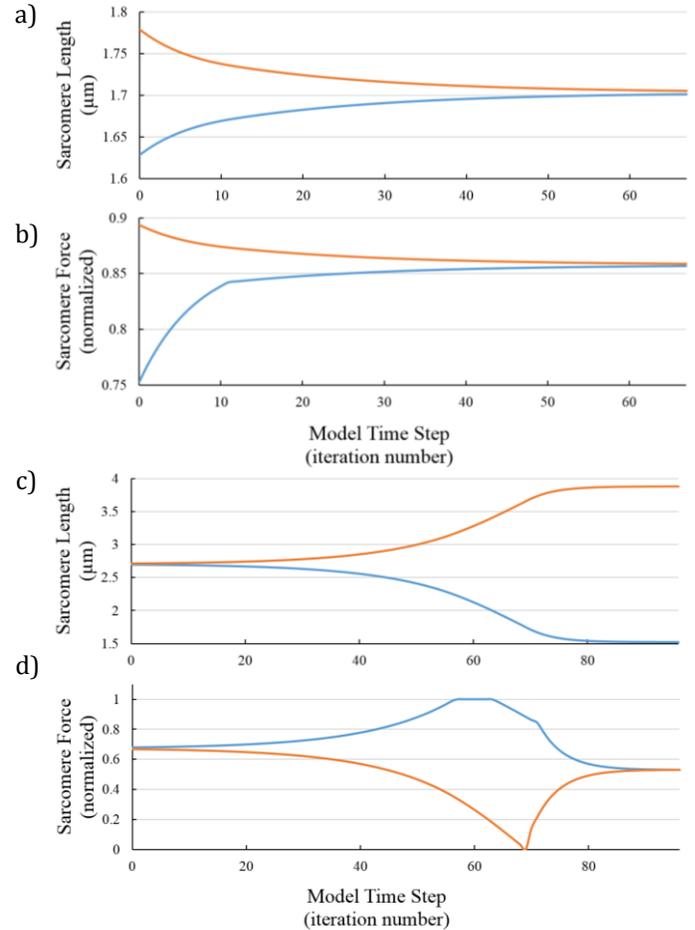


Figure 5: Simulation results for two independent sarcomeres. The ascending limb region simulation of sarcomere lengths (a) and forces (b) over time shows stability while the descending limb region simulation of sarcomere lengths (c) and forces (d) over time shows instability.

to 2.6-2.8 μm resulted in stability with uniform length. Equilibrium for all sarcomeres was reached with lengths at 2.70 μm and a normalized force of 1.44 (Fig. 7a and Fig. 7b).

Setting C_1 to 0.2 and initial sarcomere lengths to 2.6-2.8 μm resulted in stability with non-uniform length. Equilibrium for the sarcomeres was reached with the lengths ranging between 1.65-3.56 μm and a normalized force of 0.80 (Fig. 7c and Fig. 7d).

Setting C_1 to 0.3 and initial sarcomere lengths to 2.6-2.8 μm resulted in instability with non-uniform length. Equilibrium for the sarcomeres was reached with lengths at either 2.00 μm or 3.39 μm at a normalized force of 0.99 (Fig. 7e and Fig. 7f).

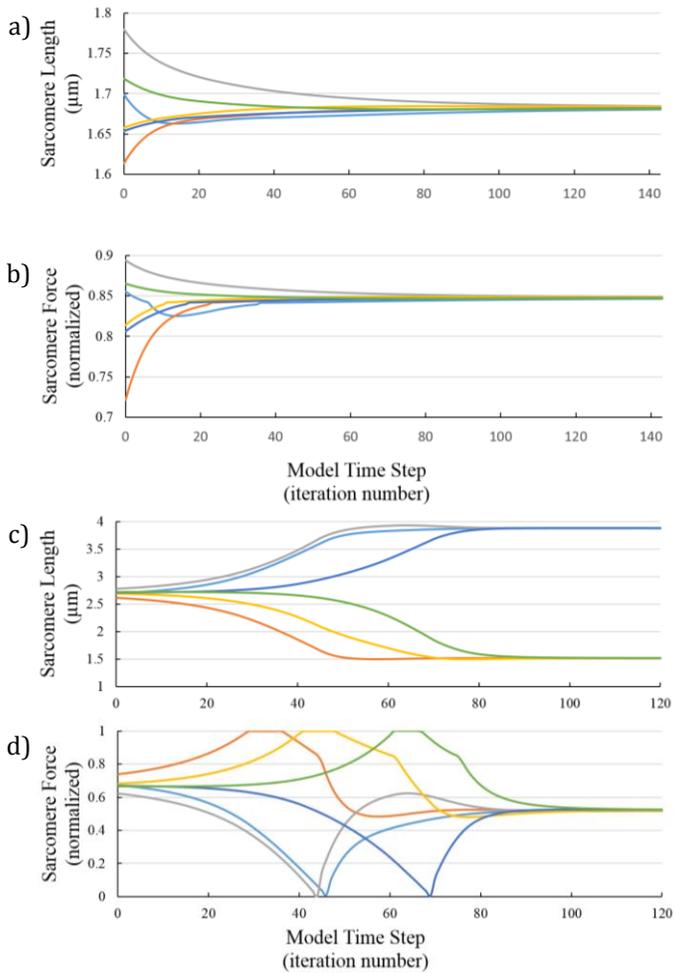


Figure 6: Simulation results for six independent sarcomeres. The ascending limb region simulation of sarcomere lengths (a) and forces (b) over time shows stability while the descending limb region simulation of sarcomere lengths (c) and forces (d) over time shows instability.

Passive Force Shifted Towards Shorter Sarcomere Lengths

With twenty dependent sarcomeres in series, setting C_1 to -0.15 and initial sarcomere lengths to $2.6\text{--}2.8\ \mu\text{m}$ resulted in instability with non-uniform length. Equilibrium for the sarcomeres was reached with lengths at either $1.68\ \mu\text{m}$ or $3.71\ \mu\text{m}$ at a normalized force of 0.84 (Fig. 8a and Fig. 8b). Setting C_1 to -0.25 and initial sarcomere lengths to $2.6\text{--}2.8\ \mu\text{m}$ resulted in stability with non-uniform length. Equilibrium for the sarcomeres was reached with the lengths ranging between $2.03\text{--}3.97\ \mu\text{m}$ and a normalized force of 1.23 (Fig. 8c and Fig. 8d). Setting C_1 to -0.35 and initial sarcomere lengths to $2.6\text{--}2.8\ \mu\text{m}$ resulted in instability with non-uniform length. Equilibrium

for the sarcomeres was reached with the lengths ranging between $1.60\text{--}4.33\ \mu\text{m}$ and a normalized force of 1.86 (Fig. 8e and Fig. 8f).

Discussion

Sarcomeres Independent of Adjacent Sarcomeres

The purpose of developing the models of independent sarcomeres was to lay the foundations of the model and verify the instability of the descending limb based on current theory. The two sarcomeres in series allowed for easy model development and to show that there was stability on the ascending limb and instability on the descending limb of the force-length relationship. This model was successfully implemented and the results were as expected. The six sarcomere model was implemented to test the generalization of two sarcomere model to an “n” sarcomere model scenario. Models were tested for up to 100 sarcomeres in series and produced the same results as the six sarcomere model, that is, instability on the descending limb region.

Sarcomeres Dependent on Adjacent Sarcomeres

Determining any biological significance of these solutions requires an understanding of the models. The simulations illustrated that there exist mathematical solutions where the sarcomeres are stable and, at equilibrium, have uniform lengths, stable with non-uniform lengths, and unstable. These different outcomes depend on the constant C_1 , which is the linear scaling factor used for the shifting of the passive force. C_1 has a different effect depending on whether there is a right (passive force is shifted towards longer sarcomeres) or left (towards shorter sarcomeres) shift.

Passive Force Shifted Towards Longer Sarcomere Lengths

Decreasing C_1 minimizes the contribution of the shifting passive force has on the total sarcomere force, and the passive force remains further to the left on the force-length relationship. If C_1 is too low, as with the simulation using $C_1=0.1$, the passive force is too large to have

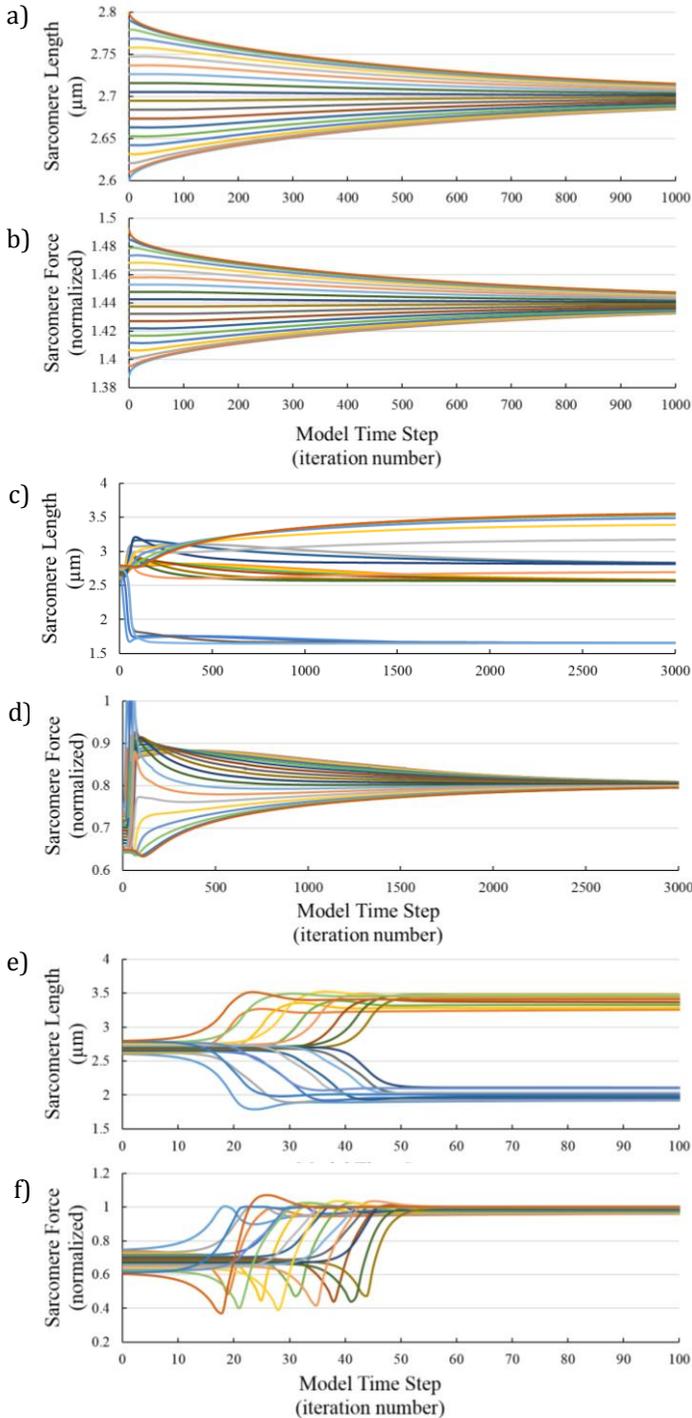


Figure 7: Simulation results for twenty dependent sarcomeres with passive force shifting to the right with constants $C = 0.1$ with sarcomere lengths (a) and forces (b) over time, $C = 0.2$ with sarcomere lengths (c) and forces (d) over time, $C = 0.3$, with sarcomere lengths (e) and forces over time (f).

biological significance. If C_1 is too high, as with the simulation using $C_2 = 0.3$, the passive force becomes too small resulting in instability

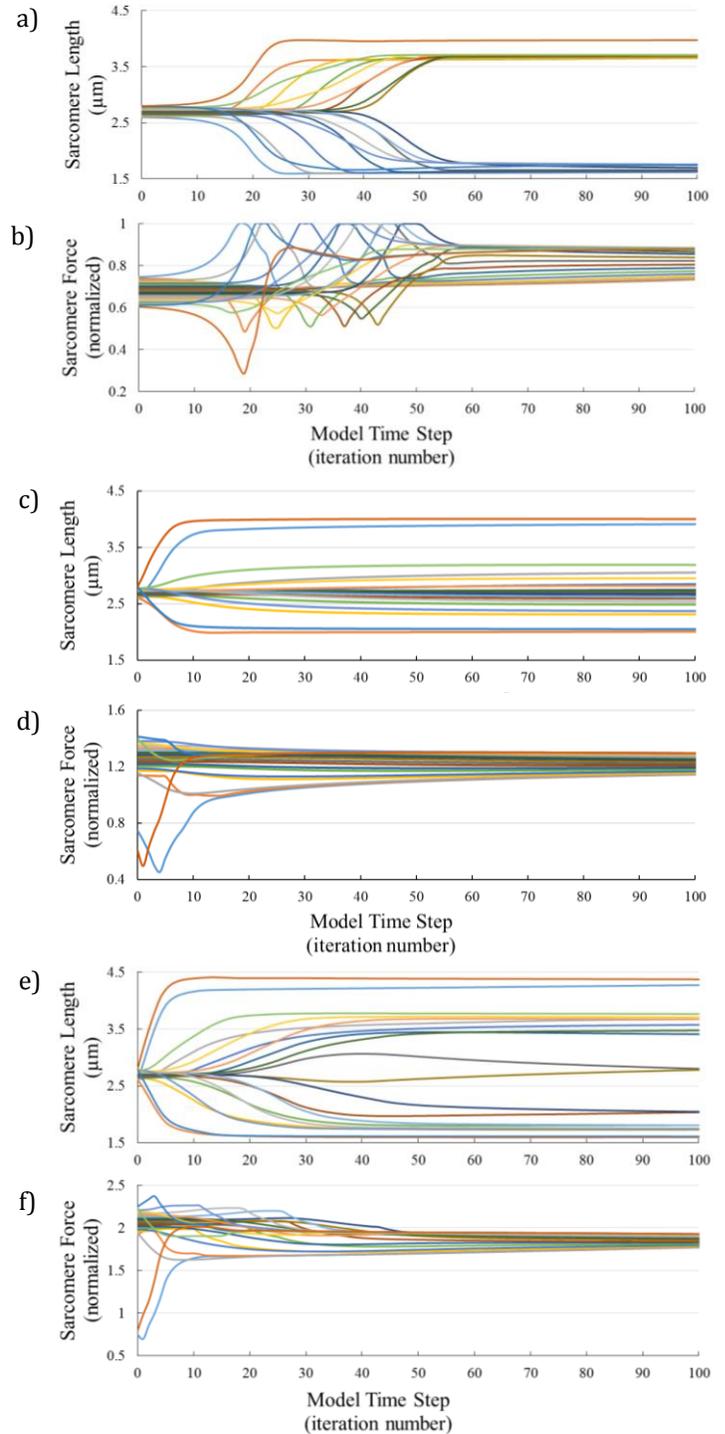


Figure 8: Simulation results for twenty dependent sarcomeres with passive force shifting to the left with constants $C = -0.15$ with sarcomere lengths (a) and forces (b) over time, $C = -0.25$ with sarcomere lengths (c) and forces (d) over time, $C = -0.35$, with sarcomere lengths (e) and forces over time (f).

due to the active forces. However, if C_2 is chosen to balance passive and active forces as with $C_2 = 0.2$, then stability is reached and sarcomere lengths at equilibrium are non-uniform. This

theoretical result matches that observed experimentally in single myofibrils [4].

Mathematically, this result is explained as follows: if *sarcomeres* $i \pm 1$ are longer than *sarcomere* i , then *sarcomere* i would have its passive force shifted far to the right, decreasing its passive force. On the descending limb, *sarcomere* i would have a larger active force. This balance between active and passive force creates stability.

Biologically, this result may be interpreted as follows: some mechanism of stretching an adjacent sarcomere causes the passive force of the sarcomere to decrease. Speculatively, there could be some interconnecting structure, a filamentous protein for example, that could achieve the desired interrelation between adjacent sarcomeres. Conclusively, if this interconnecting relation exists, then there is a mathematical solution that creates stability at non-uniform lengths on the descending limb region of the force-length relationship.

Passive Force Shifted Towards Shorter Sarcomere Lengths

Decreasing C_1 minimizes the contribution of the shift of passive force on the total (active and passive) sarcomere force. For this scenario, the passive force remains further to the right on the force-length relationship.

If C_1 is too low, as with the simulation with $C_1 = -0.15$, then the passive force is too small and instability similarly to the independent sarcomere models occurs. If C_1 is too large, as with the simulation with both $C_1 = -0.25$ and $C_1 = -0.35$, then the passive force becomes too large to have biological significance. It is noted that even though the forces are too high, stability with non-uniform lengths can be achieved on the descending limb region.

Mathematically, if *sarcomeres* $i \pm 1$ are longer than *sarcomere* i , then *sarcomere* i would have its passive force shifted far to the left, increasing its passive force. However, *sarcomeres* $i \pm 1$ still have a larger passive force. On the descending limb, *sarcomere* i would have a larger active force. Therefore, this balance between active and passive forces results in stability. The distribution

of sarcomere lengths along the myofibril oscillates symmetrically similar to $f(x) = x \cdot \sin(x)$. Similar to the right shift of the passive force, there exists a mathematical solution with stability at non-uniform lengths on the descending limb region of the force-length relationship. However, the total force generated is too high to have biological significance.

Summary

Overall, the independent models behaved as expected with the instability on the descending limb of the force-length relationship. Between the two dependent models, the passive force shifted towards longer sarcomere lengths (i.e. constant C_1 is positive), proved to be a better model because both stability was achieved in the descending limb of the force-length relationship, and the forces produced by each sarcomere was more reasonable (under a value of 1).

Conclusion and Future Work

Sarcomeres in series are theoretically predicted to be unstable on the descending limb region of the force-length relationship. However, experimental evidence has shown that sarcomeres can be stable on the descending limb region, typically with small amounts of non-uniformity between the sarcomeres [4]. The hypothesis was to test the long-held assumptions that sarcomeres are independent units of force generators by developing computational models of dependent sarcomeres in series. The major finding of these models was that mathematically, sarcomeres with a variable passive force can reach equilibrium at different lengths from each other on the descending limb of the force-length relationship. The next stages in this work is to continue developing models for sarcomeres in series. First, a variable passive force slope change will be implemented, which varies the slope of the passive force rather than the sarcomere length at which passive forces is engaged. Then, various initial conditions need to be implemented to further test the models that have already been developed, such as random initial lengths or initial lengths following a normal distribution. Once these models have been developed, they can

be interpreted mathematically, mechanically and physiologically.

Acknowledgements

I would like to thank the Human Performance Lab for their support and resources in completing this project.

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