



Force–length relation of skeletal muscles: from sarcomeres to myofibril

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Received: 15 May 2018 / Accepted: 16 July 2018 / Published online: 26 July 2018
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Abstract

Sarcomeres are building blocks of skeletal muscles. Given force–length relations of sarcomeres serially connected in a myofibril, the myofibril force–length relation can be uniquely determined. Necessary and sufficient conditions are derived for capability of fully lengthening or completely shortening a myofibril under isometric, eccentric or concentric contraction, and for the myofibril force–length relation to be a continuous single-valued function. Intriguing phenomena such as sarcomere force–length hysteresis and myofibril regularity are investigated and their important roles in determining myofibril force–length relations are explored. The theoretical analysis leads to experimentally verifiable predictions on myofibril force–length relations. For illustration, simulated force–length relations of a myofibril portion consisting of a sarcomere pair are presented.

Keywords Force–length relation · Sarcomere inhomogeneity · Myofibril regularity · Hysteretic property

1 Introduction

The hierarchy of skeletal muscles runs from the top to bottom as muscles, fibre bundles, fibres, myofibrils and sarcomeres. The well studied structures of these elements indicate sarcomeres' role as building blocks of skeletal muscles responsible for force generation causing movements of body segments of animals and humans. Over more than a half-century, advances in experimental technology had brought tremendous opportunities in exploring microstructures of skeletal muscles and their self-regulatory mechanisms. It is now possible to experimentally study functionality of a myofibril even down to the level of a half sarcomere (Rassier 2012; Minozzo et al. 2013). Technological advances call upon sophistication of theoretical studies into bio-mechanical properties such as muscle force–length relations at different microscopical levels in order to gain full understanding of mechanisms of the sarcomere-based self-regulatory contractile system.

Underlining the mechanism of muscle force generation, the sliding filament theory and the Gordon–Huxley–Julian (GHJ) sarcomere force–length (F–L) relation (Gordon et al. 1966) have been overwhelmingly accepted in muscle physiology due to vast evidence gained from microscopical

structure studies. Despite reasonably good fitting of the scaled sarcomere F–L relation with experimental data of fibres and muscles (see, e.g. Gollapudi and Lin 2009; Winters et al. 2011), given complexity of sarcomere mechanics in striated muscles (Pollack 1983; Rassier 2017), precise association of the sarcomere F–L relation with that of muscles requires rigorous theoretical studies.

Apart from variations of experimental conditions and sample preparations, experimentally observed eccentric force enhancement (Edman 2012; Rassier 2012; Koppes et al. 2013; Minozzo and Lira 2013) and concentric force depression (Joumaa and Herzog 2010; Trecarten et al. 2015) in comparison with the isometric force have added difficulties to comprehension of muscle F–L relations. A line of research in sarcomere physiology focuses on causes of force enhancement and depression, which started over more than a half-century ago and has produced a long-lasting debate continued in the recent discussions (Herzog and Leonard 2013; Edman 2013).

An essential step towards the ultimate goal of discovering mechanisms of muscle force generation is to associate the myofibril F–L relation with those of the sarcomeres. In consideration of stretching an activated myofibril at a constant speed, a hypothesis put forward by Morgan (1990) suggests the occurrence of the 'popping' phenomenon, namely sarcomeres passing plateaus and descending regions of their F–L curves, one at a time and from the weakest to the strongest sarcomere. Figure 1 illustrates this hypothesis along

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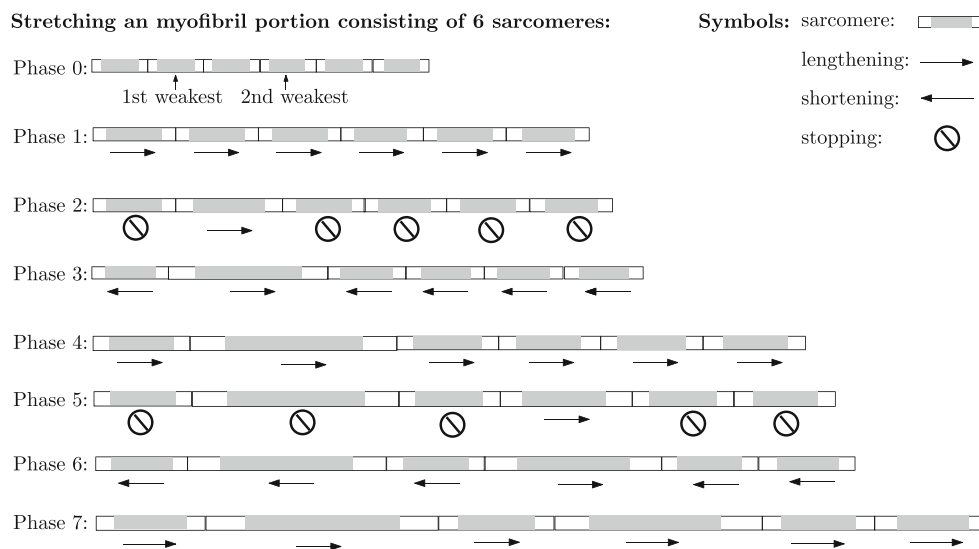


Fig. 1 Illustration of ‘popping’, ‘resisting’ and ‘yielding’ phenomena

with a few extra details of the phenomenon to be examined in this current paper. The temptation of applying the ‘popping’ hypothesis to interpret force enhancement in eccentric contraction is huge, whilst its importance in its own right has been largely overlooked. As will be proved in this current study, caused by the existence of force descending region and inhomogeneity of sarcomeres, the ‘popping’ and also its reverse can occur in a myofibril under any of the three contractile conditions. Moreover, their occurrences are considerably helped by sarcomere eccentric force enhancement and concentric force depression.

Some evidence of the ‘popping’ hypothesis appears to be the ‘resisting’ and ‘yielding’ phenomena observed in sarcomeres’ F–L responses to rapid myofibril stretch (Shimamoto et al. 2009). In Fig. 1, with respect to every individual sarcomere, status of stopping and shortening correspond to the ‘resisting’, and the thereafter lengthening to ‘yielding’ phenomena. Under consecutive discrete stretches, which is the condition to be assumed in the current study, increased sarcomere length inhomogeneity has been observed in a myofibril portion (Rassier 2012), which evidence the general ‘popping’ phenomenon. More recently, this phenomenon was also observed under isometric and eccentric contractions in a systematic experimental study (Johnston et al. 2016).

Understanding sarcomeres’ behaviours in the force descending regions of their F–L curves under different contractions is the key for comprehension of myofibril F–L relations. By assuming non-overlap between active and passive sarcomere F–L curves, in a primary theoretical study of myofibril F–L relations (Allinger et al. 1996), the standard GHJ model was considered inappropriate for sarcomeres to generate experimentally comparable myofibril F–L relations. However, the

non-overlap assumption is unrealistic and rarely adopted in studies of muscle physiology.

To achieve the ultimate goal of deducing the muscle F–L relation from those of sarcomeres, this investigation focuses on establishment of a quantitative relationship between sarcomere and myofibril F–L relations. Sarcomere inhomogeneity, force enhancement and depression will be considered in determination of myofibril F–L relations. With a rigorous treatment involving elementary function analysis only, the analysis is general and applies to both stretching and shortening of myofibrils under different contractile conditions. The result is also illustrated by a numerical simulation.

2 Methods

The F–L relation of a sarcomere or a myofibril is an external mechanical property of the object, and only static F–L relations are considered in this study. As evidenced by vast experiments reported in the literature, a change in the contractile force due to a length change endures a transit period but can settle eventually. This ensures relevance and importance of this theoretical study.

2.1 Sarcomere F–L relation

An ideal sarcomere has the shape of a right circular cylinder with a length of a few microns and diameter of a portion of its length. Among several empirical F–L models which have shown in various degrees of agreement with experimental data, the GHJ model stands out and is adopted in this study due to its wide recognition as a consequence of well fitting with the sliding filament theory. Development of a theoretical sarcomere F–L model from first principles

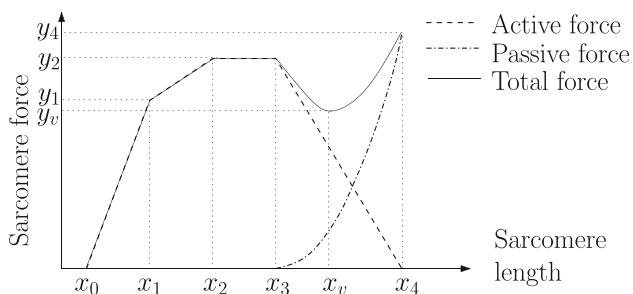


Fig. 2 Active/passive/full sarcomere F–L diagrams

would be extremely difficult because of profound complexity in microstructures and bio-chemical reactions in sarcomeres. However, some important advances have been made in theoretical modelling of muscle contractile mechanisms (see, e.g. Stålhand et al. 2008, 2016; Vita et al. 2017).

2.1.1 Gordon–Huxley–Julian model

Proposed for the active F–L relation for sarcomeres under isometric contraction, the GHJ model is a diagram illustrated in Fig. 2 (dashed line). Active force y_a is a piecewise linear function of sarcomere length x , denoted by $y_a = f_a(x, q_a)$ with

$$f_a(x, q_a) = a_k x + b_k, \quad x \in [x_{k-1}, x_k] \tag{1}$$

for $k = 1, \dots, 4$, where coefficients a_k and b_k are specified by the parameters $q_a = \{x_0, \dots, x_4, y_1, y_2\}$ as

$$a_1 = \frac{y_1}{x_1 - x_0}, \quad a_2 = \frac{y_2 - y_1}{x_2 - x_1}, \quad a_3 = 0, \quad a_4 = -\frac{y_2}{x_4 - x_3},$$

$$b_1 = -a_1 x_0, \quad b_2 = y_1 - a_2 x_1, \quad b_3 = y_2, \quad b_4 = y_2 - a_4 x_3.$$

Outside range $[x_0, x_4]$, $f_a(x, q_a)$ is undefined and not considered because being shorter than x_0 would not be resulted from sarcomere’s contraction, and longer than x_4 could cause irreversible damage of normal functionality to the sarcomere.

2.1.2 Passive F–L relation

As illustrated in Fig. 2 (dashed-point line), a widely adopted passive sarcomere F–L relation is an exponential function (Zajac 1989), denoted by $y_p = f_p(x, q_p)$ with

$$f_p(x, q_p) = p_1 \left(e^{p_2(x-x_p)} - 1 \right), \quad x \in [x_p, x_4], \tag{2}$$

and $q_p = \{x_p, x_4, p_1, p_2\}$, where $x_p = x_3$. It is assumed that the maximal passive force is not less than the maximal active force, namely $y_4 = f_p(x_4, q_p) \geq y_2$. Function (2) is not defined outside $[x_p, x_4]$. Taking assumption $x_p = x_3$ is just for clarity of the analysis to follow, whilst the use of the

axillary symbol x_p leaves room for consideration of the case $x_p \neq x_3$.

2.1.3 Full F–L relation

Induced in a sarcomere is the total force (active and passive) as illustrated (solid line) in Fig. 2. Based on (1) and (2), the (full) F–L relation $y = f(x, q)$ is expressed compactly as

$$f(x, q) = a_k x + b_k + H(\tilde{x}_p) p_1 \left(e^{p_2 \tilde{x}_p} - 1 \right),$$

$$x \in [x_{k-1}, x_k] \tag{3}$$

for $k = 1, \dots, 4$, where $q = \{x_0, \dots, x_4, x_p, y_1, y_2, p_1, p_2\}$, $\tilde{x}_p = x - x_p$, and $H(z)$ is the Heaviside function, namely $H(z) = 1$ for $z \geq 0$ and $H(z) = 0$ for $z < 0$.

Setting gradient $\frac{dy}{dx} = 0$ for $x > x_3$ yields the solution of x as $x_v = x_3 + \frac{p_3}{p_2}$ with $p_3 = \ln \left(\frac{y_2}{p_1 p_2 (x_4 - x_3)} \right)$. Clearly if and only if $x_v \in (x_3, x_4)$, a force valley exists with minimal force $y_v = f(x_v, q)$. Typically, a F–L curve has one plateau over $[x_2, x_3]$, two ascending regions, one over $[x_0, x_2]$ and the other over $[x_v, x_4]$, and one descending region over $[x_3, x_v]$. The F–L curve (solid line) in Fig. 2 is illustrative with $y_v < y_1$. It is possible $y_v > y_1$ for a different F–L curve.

Function $y = f(x, q)$ is continuous and single-valued, whilst its inverse is continuous but normally not single-valued. The notion of the inverse, denoted by $x = f^{-1}(y, q)$, is useful although it does not usually have an explicit expression.

2.1.4 Hysteretic behaviours

Under different types of contraction, the forces induced in a sarcomere at a given length are normally different, observed as eccentric force enhancement (Edman 2012; Rassier 2012) and concentric force depression (Joumaa and Herzog 2010; Trecarten et al. 2015) in comparison with the isometric force. This suggests the existence of three sarcomere F–L curves corresponding to the three contractile types, and hence implies a hysteretic nature of sarcomere F–L behaviours (Wilson et al. 1970; Paterson et al. 2010). As illustrated in Fig. 3, the eccentric and concentric F–L relations are represented by one-directional curves, whilst the isometric F–L curve is bidirectional (not indicated to have a clear overall appearance). A change between eccentric and concentric contractions makes the sarcomere transit from one F–L curve towards the other. The change can happen at any particular point (x_c, y_c) on these curves and the F–L relation during the transition is assumed to be represented by a bidirectional straight line with a fixed gradient $k_c > 0$. Figure 3 is merely illustrative and exact shapes of the curves are less important.

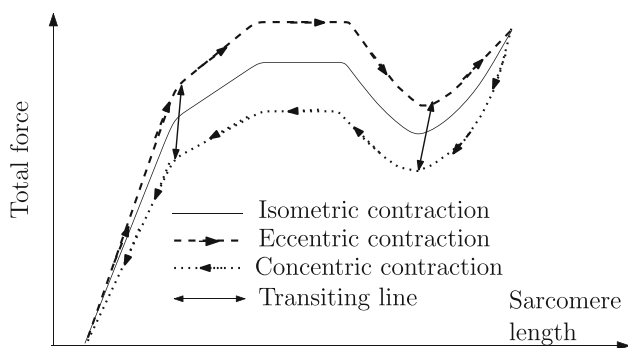


Fig. 3 Sarcomere F–L hysteresis

In consideration of F–L hysteresis and transition between F–L curves, the full sarcomere F–L relation is described by $y_s = f_s(x, p_s)$ with

$$f_s(x, p_s) = \begin{cases} f(x, \bar{q}), & \text{(eccentric/concentric/isometric)} \\ k_c(x - x_c) + y_c, & \text{(transiting)} \end{cases} \quad (4)$$

where $f(x, \bar{q})$ is defined in (3) with $\bar{q} = \{x_0, \dots, x_4, x_p, \bar{y}_1, \bar{y}_2, p_1, p_2\}$, $p_s = \{\bar{q}, k_c\}$; $x_c \in [x_0, x_4]$ and $y_c = f(x_c, \bar{q})$. Here, with (\bar{y}_1, \bar{y}_2) being replaced by (\hat{y}_1, \hat{y}_2) , $(\check{y}_1, \check{y}_2)$ or (y_1, y_2) in \bar{q} , $\bar{q} = \hat{q}$, \check{q} or q , corresponding to eccentric, concentric or isometric contraction. For $l = 1, 2$, $\check{y}_l < y_l < \hat{y}_l$.

Formula (4) postulates that eccentric and concentric F–L relations resemble the isometric one. It also implies that although sarcomere lengthening and shortening follow different active F–L curves, they have the same passive F–L relation. These assumptions impose no major restriction on generality of the study and taking them is only for simplicity and clarity of the analysis to follow.

2.2 Sarcomeres in myofibril

A myofibril or part of it contains a string of sarcomeres connected in series by Z-discs whose thickness and longitudinal flexibility are ignored in this study. Lateral forces and the associated lateral movements in a myofibril are also not considered. Making these assumptions simplifies considerably the theoretical analysis, whilst still allows exploration of the essential mechanisms of interactions among sarcomeres.

2.2.1 F–L relation of series sarcomeres

Referring to (3) and (4), the F–L relations of n individual sarcomeres can be expressed as $y_{s,i} = f_s(x_{s,i}, p_{s,i})$ with, $\forall i \in n$,

$$f_s(x_{s,i}, p_{s,i}) = \begin{cases} f(x_{s,i}, \bar{q}_{s,i}), & \text{(eccentric/concentric/isometric)} \\ k_{c,i}(x_{s,i} - x_{c,i}) + y_{c,i}, & \text{(transiting)} \end{cases} \quad (5)$$

and

$$f(x_{s,i}, \bar{q}_{s,i}) = a_{k,i}x_{s,i} + b_{k,i} + H(\tilde{x}_{p,i}) p_{1,i} (e^{p_{2,i}\tilde{x}_{p,i}} - 1), \quad (6)$$

where sarcomere length $x_{s,i} \in [x_{k-1,i}, x_{k,i})$ for $k = 1, \dots, 4$, $\tilde{x}_{p,i} = x_{s,i} - x_{p,i}$, $x_{c,i} \in [x_{0,i}, x_{4,i}]$, $y_{c,i} = f(x_{c,i}, \bar{q}_{s,i})$, $\bar{q}_{s,i} = \{x_{0,i}, \dots, x_{4,i}, x_{p,i}, \bar{y}_{1,i}, \bar{y}_{2,i}, p_{1,i}, p_{2,i}\}$ and $p_{s,i} = \{\bar{q}_{s,i}, k_{c,i}\}$. In general, here it is assumed that variations in parameters $p_{s,i}$ s represent effects of sarcomere inhomogeneity on sarcomere F–L relations.

The series connectivity implies that myofibril length x_o and force y_o are associated with those of sarcomeres as $x_o = \sum_{i=1}^n x_{s,i}$ and $y_o = y_{s,i}$, $\forall i \in n$.

2.2.2 Force and length ranges

From sarcomere F–L relations, length and force ranges of a myofibril can be readily determined. To be specific, denote the ranges by $[x_{\min}, x_{\max}]$ and $[0, y_{\max}]$, respectively. First, recall the assumption that the maximal passive force of any sarcomere is not less than the maximal active force of every sarcomere, namely $y_{4,i} \geq y_{2,i}$, $\forall i \in n$. The maximal myofibril’s force is therefore the minimum of maximal sarcomere passive forces, namely $y_{\max} = y_{4,\min} = \min\{y_{4,i} : i \in n\}$. Since sarcomere $x_{s,i}$ is not defined outside $[x_{0,i}, x_{4,i}]$, $\forall i \in n$, myofibril boundaries are $x_{\min} = \sum_{i=1}^n x_{0,i}$ and $x_{\max} = \sum_{i=1}^n f^{-1}(y_{\max}, \bar{q}_{s,i})$. Clearly, $x_{\max} = \sum_{i=1}^n x_{4,i}$ when $y_{4,\min} = y_{4,i}$, $\forall i \in n$. Fully lengthening and completely shortening a myofibril are referred to as myofibril x_o reaching x_{\max} and x_{\min} , respectively.

2.2.3 Regular myofibril

As will be seen, the sarcomeres having no valleys in their F–L curves behave ordinarily during myofibril lengthening or shortening because their F–L functions are monotonically non-decreasing. As a consequence of sarcomere inhomogeneity, parameter variations in sarcomere F–L curves with valleys must nevertheless be restricted in order to have reasonable myofibril F–L behaviours, namely myofibril’s F–L relation is a continuous single-valued function. Such a myofibril can be fully stretched or completely shortened, and length and force increments can be arbitrarily small in magnitude. This leads to the concept of myofibril regularity.

To have a simple exposition of the concept, two assumptions are made. First, it is practically impossible that two force plateaus and valleys have exactly the same height and depth, respectively. Therefore, $y_{2,i} \neq y_{2,j}$ and $y_{v,i} \neq y_{v,j}$ for $i \neq j$ are assumed. Second, it is assumed that variations in force plateaus and valleys in sarcomere F–L relations due to sarcomere inhomogeneity are not beyond those caused by

sarcomere hysteresis, namely $\hat{y}_{2,\min} \geq \check{y}_{2,\max}$ is assumed, where $\hat{y}_{2,\min} = \min\{\hat{y}_{2,i} : i \in n\}$ and $\check{y}_{2,\max} = \max\{\check{y}_{2,i} : i \in n\}$. This assumption is consistent with experimentally evidenced considerable force enhancement (Edman 2012; Rassier 2012) and depression (Joumaa and Herzog 2010; Treccartan et al. 2015) in comparison with the isometric force. Situations where these two assumptions are not taken are discussed in Sect. 4.

Definition 1 A myofibril is said to be

- *weakly regular* if (a) is true;
- *eccentrically/concentrically regular* if (a) and (b) are true;
- *isometrically regular* if (a), (b) and (c) are true;

where the three scenarios are specified as:

- (a) no sarcomere’s force plateau exceeds the minimum of maximal passive forces of all sarcomeres;
- (b) if the gradient of sarcomere’s F–L relation is negative at a particular length, so is that of myofibril’s F–L relation at an associated length;
- (c) heights of sarcomere force plateaus and depths of sarcomere force valleys are in the same order.

The specifications of (a) and (b) are clear. Scenario (c) implies that if a sarcomere’s force plateau is higher than another sarcomere’s force plateau, the depths of their force valleys must be in that order as well.

2.3 Methodology

Given the sarcomere F–L relations defined in (5), the myofibril F–L relation can be determined by solving the n equations

$$x_o = \sum_{i=1}^n x_{s,i}, \quad f_s(x_{s,i}, p_{s,i}) = f_s(x_{s,i+1}, p_{s,i+1}), \quad i = 1, \dots, n - 1 \tag{7}$$

for all sarcomere lengths $x_{s,i}$ with a given myofibril length $x_o \in [x_{\min}, x_{\max}]$. Once (7) is solved, myofibril force y_o is readily obtained from $y_o = f_s(x_{s,i}, p_{s,i})$ for any i . It is desirable but normally impossible to have an explicit solution for all $x_{s,i}$ and hence in general an explicit expression for the myofibril F–L relation $y_o = f_o(x_o, q_o)$ does not exist. Use of the second equation in (7) implies consideration of the static myofibril F–L relation and hence sarcomeric inertia effects are ignored. Clearly, only if myofibril dynamics are stable, which is assumed in the analysis undertaken, this consideration is adequate.

Since a sarcomere force may correspond to more than one sarcomere length, the myofibril F–L relation based on those

of sarcomeres becomes particularly traceable if the given x_o in (7) takes in turn values from a sequence. For example, $x_{\min} + (i - 1)\delta x_o$ and $x_{\max} - (i - 1)\delta x_o$ for $i = 1, 2, \dots, m$ with increment $\delta x_o = (x_{\max} - x_{\min}) / (m - 1)$ correspond practically to lengthening a myofibril consecutively from its minimum to its maximum, and shortening it conversely, under any specific contraction. When a myofibril is continuously activated at a given level, these two sequences correspond, respectively, to myofibril eccentric and concentric contractions. In the case of isometric contraction, a particular length of the relaxed myofibril is set first, and then the myofibril is activated to a pre-specified level.

The lengthening and shortening processes can be considered two thought experiments on a myofibril held by microneedles with a micromanipulator to have incremental elongation or reduction of myofibril’s length. The processes must be discrete since static F–L relations are considered. That is, between every two consecutive myofibril length increments, sufficient time should be given to allow the transit force to settle. This implies a practical assumption that a steady-state myofibril force can always be reached at any given myofibril length within its range. As will be proved, these sarcomere length increments can be arbitrarily small in magnitude if and only if the myofibril is regular.

The concept of length increments also facilitates numerically solving (7) since the sarcomere lengths at the previous step can be used as initial values for the current sarcomere lengths in computations. When solving (7) under myofibril eccentric or concentric contraction, it is necessary to know which of the two expressions for $f_s(x_{s,i}, p_{s,i})$ in (5) should be used. This requires to determine a transiting condition and specify starting point $(x_{c,i}, y_{c,i})$ of the transiting line. Under the assumptions stated in Sect. 2.2.3, the proof of the main results in Appendix indicates that in myofibril eccentric contraction, when sarcomere i is lengthening in its force valley region, namely $x_{s,i} \in (x_{3,i}, f^{-1}(\hat{y}_{2,i}, \hat{q}_{s,i}))$, the F–L relation of sarcomere j for $j \neq i$ is governed by the transiting line starting at $y_{c,j} = \hat{y}_{2,i}$, and $x_{c,j} = f^{-1}(\hat{y}_{2,i}, \hat{q}_{s,j})$. Similarly, in myofibril concentric contraction, when sarcomere i is shortening in the region including its force descending region, force plateau and part of its first force ascending region, namely $x_{s,i} \in (f^{-1}(\check{y}_{v,i}, \check{q}_{s,i}), x_{v,i})$, sarcomere j for $j \neq i$ follows the transiting line starting at $y_{c,j} = \check{y}_{v,i}$, and $x_{c,j} = f^{-1}(\check{y}_{v,i}, \check{q}_{s,j})$. In the both cases, $x_{c,j}$ can be uniquely determined because sarcomere j is at a known length when sarcomere i enters its force descending region.

3 Results

To appreciate generality and limitation of the results presented in this section, the assumptions made so far on sarcomere F–L relations are summarised below.

- A1 The GHJ model represents the isometric active F–L relation.
- A2 An exponential function describes the passive F–L relation.
- A3 The maximal active force is not greater than the maximal passive force across all sarcomeres in a myofibril.
- A4 The eccentric and concentric F–L relations are obtained by scaling up and down the isometric F–L relation, respectively.
- A5 Transiting between the eccentric and concentric F–L curves follows a bidirectional straight line.
- A6 Force plateaus/valleys of different F–L relations do not have exactly the same height/depth.
- A7 The highest concentric force plateau is not greater than the lowest eccentric force plateau among all sarcomeres.
- A8 Z-line thickness and the lateral movement of sarcomeres are ignored.

Cases where assumptions A1–A7 are not taken are discussed in Sect. 4.

3.1 Passive F–L relation

The passive F–L relation of a myofibril can be deduced exclusively from passive F–L relations of sarcomeres. From the fact that myofibril’s passive force equals individual sarcomere’s passive force, namely $y_o^p = y_{s,i}^p$, and (2), sarcomere length $x_{s,i} = x_{p,i} + \frac{1}{p_{2,i}} \ln\left(\frac{y_o^p}{p_{1,i}} + 1\right)$ is obtained. This leads to the myofibril’s length as a function of myofibril passive force as

$$x_o = \bar{x}_p + \sum_{i=1}^n \frac{1}{p_{2,i}} \ln(y_o^p/p_{1,i} + 1), \quad y_o^p \in [0, y_{4,\min}] \quad (8)$$

where $\bar{x}_p = \sum_{i=1}^n x_{p,i}$.

If the passive myofibril F–L relation is denoted by $y_o^p = f_p(x_o, q_o)$ with parameterisation $q_o = \{p_{1,1}, p_{2,1}, \dots, p_{1,n}, p_{2,n}, \bar{x}_p\}$, (8) represents its inverse, but not the function itself whose explicit expression is desirable but normally unobtainable. In some special cases, for instance, if the two exponential coefficients are the same ($p_{2,1} = p_{2,2} = p_2$ for $n = 2$) and the maximal passive force of sarcomere one is less than that of sarcomere two ($y_{4,1} < y_{4,2}$), the explicit inverse of (8) is

$$y_o^p = -\frac{1}{2}(p_{1,1} + p_{1,2}) + \frac{1}{2}\sqrt{(p_{1,1} - p_{1,2})^2 + 4p_{1,1}p_{1,2}e^{p_2(x_o - x_{p,1} - x_{p,2})}} \quad (9)$$

for $x_o \in [x_{p,1} + x_{p,2}, x_{4,1} + x_{p,2} + \frac{1}{p_2} \ln(\frac{y_{4,1}}{p_{1,2}} + 1)]$; or if the two coefficients of the exponential function are the same for all sarcomeres ($p_{1,i} = p_1$ and $p_{2,i} = p_2, \forall i$), the inverse of (8) has an explicit expression

$$y_o^p = p_1 \left(e^{p_2(x_o - \bar{x}_p)/n} - 1 \right), \quad x_o \in [\bar{x}_p, x_{\max}]. \quad (10)$$

3.2 Full F–L relation

The active myofibril F–L relation cannot be determined solely from active sarcomere F–L relations, but by subtracting the passive myofibril F–L relation from the full myofibril F–L relation. This implies the assumption of parallel arrangement of the equivalent passive and active elements in a myofibril, a type of the Hill’s muscle model (Zajac 1989).

The following proposition and its corollary with their proofs found in Appendix state necessary and sufficient conditions for the capability of a myofibril being fully lengthened/shortened and the myofibril F–L relation being a continuous single-valued function.

Proposition 1 *Under eccentric/concentric/isometric contraction, a myofibril can be fully stretched and/or completely shortened and the induced contractile force is a single-valued continuous function of the myofibril length if and only if the myofibril is eccentrically/concentrically/isometrically regular.*

Corollary 1 *A myofibril can be fully stretched or completely shortened if and only if it is weakly regular.*

To predict shapes of myofibril F–L curves based on those of sarcomeres, two conventions are adopted separately in myofibril lengthening and shortening. On the order of heights of sarcomere force plateaus and the order of depths of sarcomere force valleys, the conventions are, for $i < j, \forall i, j \in n$,

- C1 (ordered force plateaus): $y_{2,i} < y_{2,j}$;
- C2 (ordered force valleys): $y_{v,i} < y_{v,j}$.

In C2, if sarcomere i has no force valley, set $y_{v,i} = y_{2,i}, \forall i$. There is no loss of generality in adopting either of C1 and C2 since neither of them suggests that sarcomeres i and j physically appear in that order within a myofibril.

Following the proofs of Proposition 1 and its corollary, the next two propositions with the associated corollaries on F–L relations of regular and weakly regular myofibrils are easily verifiable and hence their proofs are omitted.

Proposition 2 *The F–L curve of an eccentrically/concentrically/isometrically regular myofibril has n plateaus occurring from left to right in the increasing order of i . These myofibril plateaus have exactly the same heights and widths*

Table 1 Sarcomere parameters for isometric F–L curves x_i s and $1/p_2$ are in unit μm , and y_i s and p_1 in kg/cm^2

	x_0	x_1	x_2	x_3	x_4	x_p	y_1	y_2	p_1	p_2
s-sarcomere	1.270	1.670	2.000	2.250	3.650	2.250	0.272	0.068	0.200	0.800
w-sarcomere	1.274	1.671	1.999	2.251	3.660	2.251	0.262	0.067	0.200	0.795

as those of the sarcomeres. Plateaus i and $i + 1$ of the myofibril are connected by a myofibril valley if the i th sarcomere F–L curve has a valley and the myofibril valley has the same depth as that of sarcomere i , or otherwise by a curve with monotonically increasing gradients.

Running of index i in Proposition 2 leads to an extra plateau $n + 1$ which is virtual at the maximal passive myofibril force.

Corollary 2 *The eccentric F–L curve of a weakly regular myofibril has the same shape as that of an eccentrically regular myofibril, except that there is a force jump discontinuity in the region of at least one of the plateau-separating valleys and such a myofibril valley may be shallower than the corresponding sarcomere’s valley.*

Corollary 3 *The concentric F–L curve of a weakly regular myofibril has the same shape as that of a concentrically regular myofibril, except that there is a force jump discontinuity in the region between the bottoms of at least two consecutive valleys and with possible disappearance of the plateau between the two valleys.*

Corollary 4 *Under isometric contraction, a weakly regular myofibril has two F–L curves, respectively, for myofibril lengthening and shortening. The two curves have the same shape as that of an isometrically regular myofibril, except that at least one length and/or force jump occurs in a region where only a force jump occurs for a weakly regular myofibril under eccentric or concentric contraction.*

3.3 Illustrations

Consider a tiny portion of a regular myofibril consisting of just two sarcomeres, a weak one (w-sarcomere) and a strong one (s-sarcomere). Referring to the notation in (3), parameters of the sarcomere F–L relations are given in Table 1. The parameters of the active F–L relation for the strong sarcomere under isometric contraction are taken from Gordon et al. (1966), whilst for the passive F–L relation, $x_p = x_3$, and p_1 and p_2 are manually chosen so that the full F–L curve has a reasonable shape. The parameters of the weak sarcomere are generated randomly from the normal distribution with the corresponding value of the strong sarcomere as the mean and one of hundredth of the mean as the standard deviation. The associated parameters for sarcomere F–L relations under

eccentric and concentric contractions are obtained by scaling, respectively, up and down by 20% of the isometric y_1 and y_2 values.

Software tool Matlab® 2015b has been used in the coding for solving the equations in (7). Particularly, for a given sequence of myofibril length which is the sum of the two sarcomere lengths, the sarcomere lengths are obtained by determining one sarcomere length at each step through minimising the squared difference between the two F–L relations in (7) over a small range around its length.

Figure 4 shows sarcomere and myofibril forces produced by different contractions at different sarcomere and myofibril lengths, and also sarcomere length changes with respect to the myofibril length change. The horizontal axis quantifies sarcomere and myofibril lengths, whilst the vertical axis on the left quantifies forces and that on the right indicates sarcomere length. Based on the GHJ model, sarcomere/myofibril force (N) is converted from tension (kg/cm^2) by multiplying with the gravitational acceleration ($9.81 \text{ m}/\text{s}^2$) and the myofibril cross-sectional area ($8.659 \times 10^{-7} \text{ cm}^2$) calculated from the frog myofibril mean diameter ($1.05 \mu\text{m}$) (Mobley and Eisenberg 1975). The myofibril length changes in the range [2.544, 7.310] with increment 0.01. For easy comparison, the main curves in Fig. 4a–c are reproduced in 4d.

All these plots indicate that the heights and widths of sarcomere force plateaus and the depths of sarcomere force valleys are faithfully reflected on the myofibril F–L curves. These curves along with the plots of individual sarcomere length changes against myofibril length changes demonstrate that the two sarcomeres traverse their force plateaus and valleys one by one. Also, under isometric contraction, if one sarcomere is lengthening or shortening in its force descending region, the other is shortening or lengthening in its own first or second force ascending region. However, if the myofibril portion is under eccentric or concentric contraction, the other sarcomere will be shortening or lengthening on the transiting line. In the force descending region, the active myofibril F–L curves do not resemble the original sarcomere active F–L curves. In Fig. 4c, they look alike in that region because sarcomere valleys are very shallow.

As expected in Fig. 4a, during myofibril lengthening and shortening under isometric contraction, myofibril forces follow exactly the same myofibril F–L curve, and the associated sarcomere length changes follow exactly the same traces, respectively. In Fig. 4b, two cases for $\alpha = 2, 10$ are shown, where $\alpha = k_c/k_b$ with k_c being the gradient of the transiting

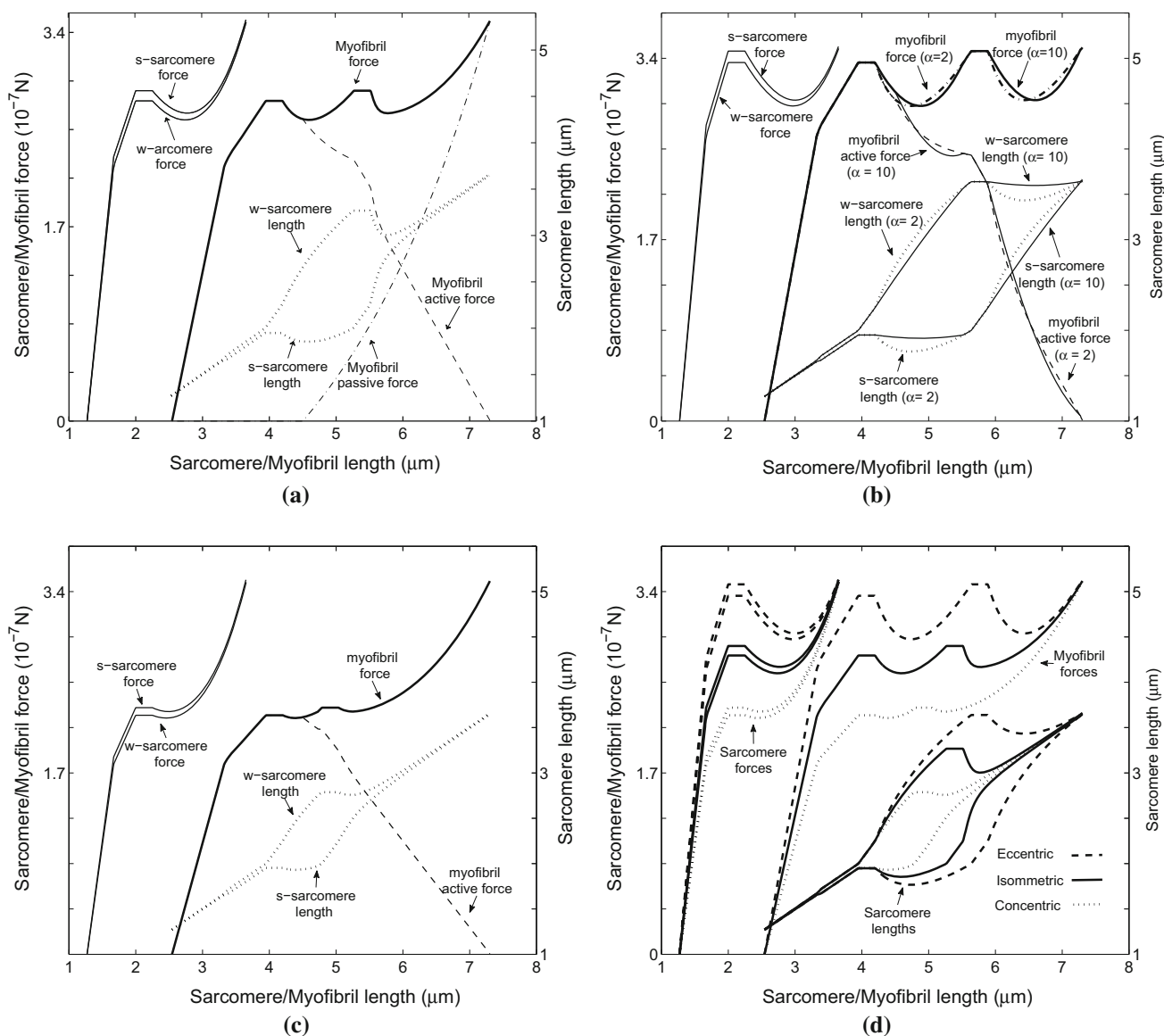


Fig. 4 Illustrative F–L curves and sarcomere length changes under different contractions (Sarcomere/myofibril forces are quantified by the vertical axis on the left, and sarcomere lengths by the vertical axis on the right). **a** Isometric curves, **b** Eccentric curves, **c** concentric curves, **d** Combined curves

line, and k_b the maximal absolute value of the gradient of strong sarcomere’s F–L curve in its force descending region. These two cases indicate that a stiff transiting line leads to less sarcomere length reduction during myofibril lengthening, as expected. Logically, a similar phenomenon (not shown in Fig. 4c) happens in the case of concentric contraction.

For clarity, only a tiny portion of a myofibril with just two sarcomeres has been considered in the numerical simulation. Due to the nature of sarcomeres passing their force plateaus and valleys one by one during myofibril lengthening or shortening, it is clear that a longer myofibril will not show any diverse F–L behaviours other than having an increased number of myofibril plateaus and valleys due to inclusion of additional sarcomeres in the myofibril.

4 Discussion

A quantitative relationship between myofibril and sarcomere F–L relations has been established in this study. The following interpretations and discussions highlight significance of the findings and implications of the assumptions made.

4.1 One-by-one passing

The theoretical analysis in this paper suggests that length changes in sarcomeres during myofibril elongation or shortening under any of the three contractions have some regular patterns. For illustration, the sarcomere with the lowest force

plateau is referred to as the ‘weakest’ sarcomere, and the one with the highest force plateau the ‘strongest’ sarcomere.

By myofibril lengthening, the weakest sarcomere will first reach and pass its force plateau, then traverse its force valley to reach its second ascending region. Meanwhile, all other sarcomeres remain in their first force ascending regions. Afterwards, the second weakest sarcomere repeats what the first weakest sarcomere did, whilst the first weakest sarcomere remains in its second force ascending region and all the remaining sarcomeres are still in their first force ascending regions. This process continues until the strongest sarcomere reaches its second force ascending region. Afterwards, the myofibril elongation proceeds to reach the maximal myofibril length with all sarcomeres lengthening in their second force ascending regions.

The reverse happens in myofibril shortening, where the strongest sarcomere goes down in its second ascending region, passes its force valley and plateau and finally reaches its first ascending region, followed by the second strongest and finally by the weakest sarcomere. Afterwards, the myofibril shortening proceeds to reach the minimal myofibril length with all sarcomeres shortening in their first force ascending regions.

During myofibril lengthening, when a sarcomere is lengthening through its force valley with force dropping first and then increasing, all other sarcomeres undergo shortening first and then lengthening. Conversely, during myofibril shortening, when a sarcomere is shortening over its force descending region, plateau and part of its first ascending region, all other sarcomeres undergo lengthening first, then stopping and finally shortening. Depending on the type of the contraction that the myofibril endures, these other sarcomeres are either on their isometric F–L curves or on the straight lines transiting between their concentric and eccentric F–L curves.

As a consequence of the theoretical analysis, the prediction on sarcomere lengthening and shortening patterns indirectly verifies the ‘popping’ hypothesis (Morgan 1990) and is indirectly evidenced by the ‘resisting’ and ‘yielding’ phenomena (Shimamoto et al. 2009) as myofibril stretching with a constant speed was considered in these previous studies. It provides a theoretical affirmation of the increased sarcomere length inhomogeneity observed through myofibril stretches (Rassier 2012). If such inhomogeneity during myofibril stretches cannot be observed (Johnston et al. 2016), certainly none of these sarcomeres contains a force descending region or their force plateaus are of a same height and width, and their valleys are of a same depth. The seemingly inter-sarcomere coordination (Shimamoto et al. 2009) ought to be a simple consequence of series connectivity of inhomogeneous sarcomeres having force descending regions.

4.2 Myofibril regularity and length/force jump

Continuity and surjection of individual sarcomere F–L functions do not guarantee the same for myofibril F–L relations under any of contractions. This paper has proved that myofibril regularity is necessary and sufficient for myofibril F–L relation being a continuous single-valued function. Roughly speaking, a myofibril is regular if it elongates with force dropping or shortens with force increasing whenever a sarcomere within it does so, and the changes in length and force can be arbitrarily small in magnitude.

For an irregular myofibril, force and/or length jump will occur during myofibril lengthening or shortening, namely a signed small myofibril length increment can lead to a large force increment, and vice versa. If a myofibril is not even weakly regular, it cannot be fully stretched or completely shortened. In that case, further lengthening may cause unrecoverable damage to the myofibril, whilst further shortening could be achieved only by other means rather than contraction. Also, remarkably an irregular myofibril may have two isometric F–L relations for lengthening and shortening, respectively. Although being largely overlapping, they have discontinuities at different locations. It appears therefore natural or at least ideal for a myofibril to be regular.

It is anticipated that, in experiments on an irregular myofibril, a force jump is to be reflected by a small length increment leading to a big force increment reading, and a length jump implies that the myofibril cannot settle to have a steady-state force reading at some particular myofibril lengths when the myofibril undergoes lengthening with a force drop or shortening with a force increase. However, it can be difficult in experiments to observe force and length jumps because theoretically these jumps are defined as non-infinitesimal which can practically be extremely small unless a grossly irregular myofibril is under examination.

The analysis of static F–L relations of sarcomeres and myofibril, as it is in this paper, is nevertheless unable to explain how a force or length jump can happen in an irregular myofibril. A myofibril force or length jump is caused by a large length increment in a sarcomere which is in its force descending region since a small length increment in this sarcomere would lead to an opposite increment in the myofibril length, contradictory to myofibril lengthening or shortening. However, in reality this sarcomere length increment has to grow from small to big and hence so does its force, which implies that an irregular myofibril may have to be shorten before lengthen or vice versa. This superficially contradicts the undergoing myofibril consecutively lengthening or shortening. Therefore, analysis of dynamics of F–L relations is required to answer the question of how force jump could happen in a static F–L relation for an irregular myofibril during lengthening or shortening, which is not pursued in this paper.

4.3 Sarcomere inhomogeneity: Good or bad?

As a natural consequence of muscle cell growth under complex conditions, sarcomere inhomogeneity is reflected in unavoidable variations of the parameters specifying sarcomere F–L relations. Complete homogeneity of sarcomeres in a myofibril would imply nonexistence of parameter variations in their F–L functions $f(x_{s,i}, \bar{q}_{s,i})$, namely $\bar{q}_{s,i} = \bar{q}_s$ for all i . Consequently, all sarcomeres have the same length as one n th of the myofibril's length, namely $x_{s,i} = x_o/n$ for all i during myofibril lengthening or shortening. This suggests that the F–L relation for a myofibril with sarcomere homogeneity is simply the sarcomere F–L relation with a length scaling, namely $y_o = f_o(x_o, q_o) = f(x_o/n, \bar{q}_s)$. Conversely, if myofibril F–L relation $y_o = f_o(x_o, q_o)$ is known, then $y_s = f_o(nx_s, q_o)$ could be considered the F–L relation of an 'average' sarcomere with n identical copies of it in the myofibril. Nevertheless, from the analysis in this paper, it is certain that this scaled myofibril F–L relation cannot have the usual shape of a Gordon–Huxley–Julian sarcomere model, namely $f_o(nx_{s,i}, q_o) \neq f_s(x_{s,i}, p_{s,i})$ for any sarcomere unless complete sarcomere homogeneity is assumed. Therefore, such an 'average' sarcomere is not a true sarcomere in any real sense.

As far as myofibril F–L relations are concerned, it is favourable to possess sarcomere inhomogeneity as partial sarcomere homogeneity could lead to uncertainties in myofibril F–L relations. Consider three cases excluded in Assumption A6: The force plateaus of two sarcomeres have: (a) the same height and width; (b) the same height but different widths; (c) as (a) but the force valleys have different depths. In cases (a) and (b), when the two sarcomeres have reached their force plateaus from the left or right ends due to myofibril lengthening or shortening, given a myofibril length increment, there are infinite many possible length increments for these two sarcomeres to have. It may not be insensible to assume that their length increments are equally half of the myofibril length increment since other sarcomeres stop lengthening or shortening, but in case (b), when the other end of the plateau with the narrow width is reached by one sarcomere, a further myofibril length increment under eccentric or concentric contraction would imply more than one possible length increments for these two sarcomeres. That is, one with zero length increment and the other with the length increment equal to that of the myofibril, or both sarcomeres with nonzero length increments and a force drop. In case (c) and under isometric contraction, assuming that the two sarcomeres are already in their force descending regions, the sarcomere with the shallower valley is able to reach its second force ascending region through myofibril lengthening, but certainly the other sarcomere is unable to do so. The uncertainties on sarcomere F–L behaviours in these cases of irregular myofibrils add further complexity to the muscle

self-regulatory mechanism and no theory seems available to explain the additional complexity.

4.4 Different types of sarcomere F–L relations

Clearly other than the Gordon–Huxley–Julian model adopted in this paper, many different types of functions such as polynomial, cubic splines, Bézier curve and asymmetric Gaussian (Mohammed and Hou 2016 and references therein) may also be used. All these models have representative features of a sarcomere F–L relation such as the maximal active and passive forces, two force ascending regions, and one descending region, which can produce similar myofibril behaviours as discussed in this paper. A simple exponential function has been adopted in this paper to represent passive sarcomere F–L relations. To focus on the key features of myofibril F–L relations, this study has not considered the possible passive force enhancement or depression (Herzog and Leonard 2002). Referring to Assumptions A1–A5, alternative active and passive F–L models, and other possible F–L relations counting for sarcomere hysteresis and transiting between eccentric, concentric or isometric F–L curves are not investigated in this paper. Nevertheless, this paper has laid a foundation for dealing with these different models.

Under the assumption of perpendicularly transversal striations of a muscle fibre, sarcomeres within such a striation can be considered in parallel connection and give rise to one stacked sarcomere. By adding up individual sarcomere F–L relations, a stacked sarcomere would have a F–L relation similar to that of a normal sarcomere, but with none or a very narrow force plateau and multiple gradient changes in other parts of the curve. Such a curve could closely resemble the asymmetric Gaussian and hence it is anticipated that the F–L relation of a fibre would show a more rounded shape than a myofibril F–L curve even if individual sarcomere F–L relations are represented by the Gordon–Huxley–Julian model which is formed by piecewise straight lines. Nevertheless, all peaks and valleys of individual groups of stacked sarcomeres should show up in a fibre F–L curve in a similar manner as the appearance of sarcomere force plateaus and valleys in a myofibril F–L curve.

4.5 Sarcomere and myofibril hysteresis

Myofibril hysteresis is a consequence of the same of sarcomeres. Sarcomere hysteresis enhances the chance of a myofibril becoming regular. As shown in this paper, the key requirement for myofibril regularity is that the sum of the reciprocals of all sarcomere F–L gradients is negative whenever the F–L gradient of one of the sarcomeres is negative. Under the assumption that none of the sarcomeres' heights is exactly the same as another, when a sarcomere is lengthening with a force drop or shortening with a force increase, other sar-

comeres must shorten or lengthen correspondingly. In such cases, in order to produce an required negative gradient for the myofibril, the positive gradients of the other sarcomeres must be as substantial as possible.

In sarcomere isometric F–L curves, positive gradients in the first force ascending regions are not particularly greater than the absolute values of the negative gradients in the force descending regions, whilst the gradient of the transiting lines between sarcomere eccentric and concentric F–L curves can be considerably substantial. Because of this, a myofibril with several hundred sarcomeres is almost certainly irregular under isometric contraction, whilst it is regular under eccentric or concentric contraction only if the gradient of the transiting lines is substantially greater than the absolute value of the negative gradient of any individual sarcomere F–L relation. For instance, if a myofibril has n sarcomeres and all the transiting lines between eccentric and concentric sarcomere F–L curves have the same gradient k_c , then the key regularity condition is reduced to $k_i < \frac{k_c}{n-1}$, $\forall i$, where $k_i = |a_{4,i} + p_{1,i} p_{2,i}|$ is the maximal absolute value of the negative gradient of sarcomere i . To have a general assessment on myofibril regularity, it would be of paramount importance carrying out experimental studies on sarcomere F–L relations during transiting between eccentric and concentric contractions.

The analysis in this paper has assumed, referring to Assumption A7, that sarcomere force variations caused by sarcomere inhomogeneity are within those caused by sarcomere hysteresis. This assumption is consistent with the evidence reported in the literature about substantial force enhancement (Edman 2012; Rassier 2012) or depression and depression (Joumaa and Herzog 2010; Trecarten et al. 2015) under eccentric or concentric contraction in comparison with the forces induced in isometric contraction. An important implication of this assumption is that a sarcomere transits towards but never reaches the next F–L curve. If this assumption is not taken, the regularity conditions for myofibrils under eccentric or concentric contraction need to be combined with more restrictive conditions for myofibril isometric regularity to cover the case of a sarcomere completing its transition between eccentric and concentric F–L curves.

5 Conclusion

In general, by lengthening or shortening a myofibril, individual sarcomeres within it will traverse, one by one and from the weakest to strongest sarcomeres or reversely, their force–length curves. This applies to a group of semi-homogeneous sarcomeres whose force plateaus have the same height and width and force valleys have the same depth. Consequently, the heights and widths of sarcomere force plateaus and the

depths of sarcomere force valleys will be preserved in the myofibril force–length curves. A myofibril fore–length curve resembles a general sarcomere force–length curve only when none of sarcomeres has a force valley or they are homogeneous.

The regularity is an ideal feature to possess for a myofibril to ensure the myofibril force–length being a continuous single-valued function. The concept of myofibril regularity can be easily extended to a muscle fibre if the fibre is assumed to be formed by stacked sarcomeres connected in series. A stacked sarcomeres consists of parallelly connected sarcomeres and its force–length relation is readily obtained from those of the sarcomeres.

It is anticipated that the myofibril irregularity will reflect itself in experiments on myofibril fore–length relations by having readings of substantial force increments with respect to small length increments and/or impossibility of having steady-state force readings at some particular myofibril lengths where the regularity conditions fail. Detailed mechanisms of force and length jumps in the force–length relations of irregular myofibrils require analysis of sarcomere force–length dynamics, which is not pursued in this paper but worthwhile to explore in the future.

Sarcomere inhomogeneity and hysteresis enhance the chance of a myofibril becoming regular. Complete sarcomere homogeneity or semi-homogeneity such as sarcomere force plateaus having the same height and width and force valleys having the same depth does not cause any problems in determination of myofibril F–L relations. Nevertheless, any additional minor sarcomere inhomogeneity will complicate myofibril lengthening or shortening behaviours. As implied by the analysis in this paper, at least for myofibril eccentric and concentric contractions, these complexities caused by minor sarcomere inhomogeneity will disappear if sarcomere force plateaus do not exist.

Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

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Appendix

The mathematical specifications of myofibril regularity conditions (a)–(c) outlined in Definition 1 are as follows.

(r1) $\bar{y}_{2,\max} \leq y_{4,\min}$;

(r2) Eccentric/concentric: $\frac{1}{a_{4,i} + p_{1,i}p_{2,i}} + \sum_{j=1, j \neq i}^n \frac{1}{k_{c,j}} < 0$, $\forall i \in S_v$;

Isometric: $\frac{1}{f'(x_{s,i}, q_{s,i})} + \sum_{j=1, j \neq i}^n \frac{1}{f'(x_{s,j}, q_{s,j})} < 0$,
 $x_{s,i} \in (x_{3,i}, x_{v,i})$, $\forall i \in S_v$;

(r3) $y_{v,i} < y_{v,j}$ if $y_{2,i} < y_{2,j}$, $j \neq i$, $\forall i, j \in S_v$.

In (r1), $\bar{y}_{2,\max}$ is $\hat{y}_{2,\max}$ (eccentric), $\check{y}_{2,\max}$ (concentric), or $y_{2,\max}$ (isometric), and $y_{4,\min} = \min\{y_{4,i} : i \in n\}$. In (r2) and (r3), S_v is the set of the sarcomeres having valleys in their F–L curves. In (r2), the expression on the left of the inequality is the reciprocal of the gradient of the myofibril F–L curve, namely $\frac{dx_o}{dy_o} = \sum_{i=1}^n \frac{1}{f'_s(x_{s,i}, p_{s,i})}$ which is piecewise continuous. With reference to the a_k -definitions in (1), $a_{4,i} = -\frac{\bar{y}_{2,i}}{x_{4,i} - x_{3,i}}$ with $\bar{y}_{2,i} = \hat{y}_{2,i}$ (eccentric) or $\check{y}_{2,i}$ (concentric); $p_{1,i}$ and $p_{2,i}$ are two coefficients of the passive F–L relation of sarcomere i ; f' is the gradient of f ; $x_{s,j} = f^{-1}(y_{s,i}, q_{s,j})$ with $y_{s,i} = f(x_{s,i}, q_{s,i})$ is in its first ascending region for $j > i$, and in its second ascending region for $j < i$ under conventions C1 or C2 stated in Sect. 3.2; The general form for condition (r2) is $\frac{1}{f'_s(x_{s,i}, p_{s,i})} + \sum_{j=1, j \neq i}^n \frac{1}{f'_s(x_{s,j}, p_{s,j})} < 0$ which is the expression for the isometric case because of $f_s(x_{s,i}, p_{s,i}) = f(x_{s,i}, q_{s,i})$, and reduced to the specific form under the two other contractile conditions with $k_{c,j}$ being the gradient of the transiting line for sarcomere j (referring to (5)).

Proof of Proposition 1

Sufficiency of (r1) to (r3) and necessity of (r1) are to be proved here, leaving necessity of (r2) and (r3) to be verified in the proof of Corollary 1.

For simplicity, let lengths x_o and $x_{s,i}$ also stand for the myofibril and sarcomere i themselves, and δx_o and $\delta x_{s,i}$ for their increments, respectively. $\delta x_o > 0$ corresponds to x_o -lengthening under eccentric or isometric contraction, whilst $\delta x_o < 0$ corresponds to x_o -shortening under concentric or isometric contraction. From the discussions in Sect. 2.2.2, the starting pair (x_o, y_o) is known. Given such a pair (x_o, y_o) , the proposition will be verified by proving that, if and only if the regularity conditions (r1) to (r3) hold, a signed $\delta x_o = \sum_{i=1}^n \delta x_{s,i}$ leads to a unique force increment δy_o over the whole range of x_o , where mutually dependent increments $\delta x_o, \delta x_{s,i}$ and δy_o can be arbitrarily small in magnitude, namely infinitesimal.

The above explains the main idea of the proof. To strike a balance between rigour/completion and simplicity, the proof considers a myofibril portion consisting of three sarcomeres

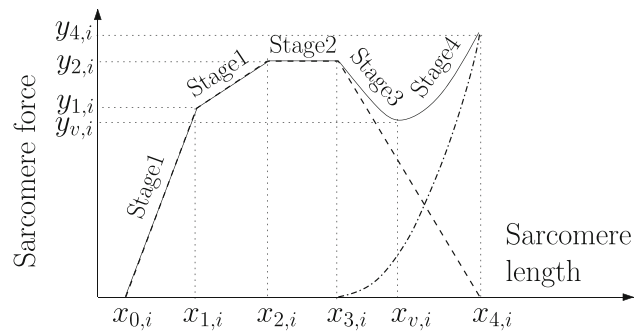


Fig. 5 Isometric F–L curve of sarcomere i with stages of lengthening or shortening

only, at the cost of omitting the formal induction step which is straightforward anyway for a general myofibril with n sarcomeres. Referring to the illustration of the ‘popping’ phenomenon in Fig. 1 and the full sarcomere F–L relation in Fig. 2, four stages of lengthening or shortening are indicated on a typical isometric sarcomere F–L relation in Fig. 5.

Lengthening x_o At the minimal length, $x_o = x_{\min} = \sum_{i=1}^3 x_{0,i}$ and $y_o = 0$. Referring to the notations in (6), define $x_{o,1} = x_{2,1} + f^{-1}(\bar{y}_{2,1}, \bar{q}_{s,2}) + f^{-1}(\bar{y}_{2,1}, \bar{q}_{s,3})$. For any infinitesimal $\delta x_o \in (0, x_{o,1} - x_{\min}]$, an infinitesimal $\delta y_o \in (0, \hat{y}_{2,1}]$ can be easily determined so that $\delta x_o = \sum_{i=1}^3 \delta x_{s,i}$ with $\delta x_{s,i} = f^{-1}(\delta y_o, \bar{q}_{s,i})$ for $i = 1, 2, 3$. This means that $x_{s,1}, x_{s,2}$ and $x_{s,3}$ can be uniquely solved from (7) for any given $x_o \in [x_{\min}, x_{o,1}]$. Hence, the proposition is verified for the case of x_o -lengthening up to $x_{o,1}$ without invoking (r1) to (r3). This corresponds to lengthening $x_{s,1}, x_{s,2}$ and $x_{s,3}$ in stage 1.

Further infinitesimally x_o -lengthening must be with $\delta y_o = 0$ through $x_{s,1}$ -lengthening in stage 2, whilst $x_{s,2}$ and $x_{s,3}$ stopping in stage 1. This means that, with any infinitesimal $\delta x_o \in (0, < x_{3,1} - x_{2,1}]$, (7) is trivially solved by $x_{s,1} = x_{2,1} + \delta x_o$ and $x_{s,i} = f^{-1}(\bar{y}_{2,1}, \bar{q}_{s,i})$ for $i = 2, 3$. Hence, the proposition is now verified for x_o -lengthening up to $x_{o,2} = x_{o,1} + x_{3,1} - x_{2,1}$, still without invoking (r1) to (r3).

If $x_{s,1}$ does not have a force valley, further x_o -lengthening implies that $x_{s,1}$ is immediately in stage 4 after leaving stage 2 without invoking (r1) to (r3). In general, if $x_{s,1}$ has a force valley, further infinitesimally x_o -lengthening must be with $\delta y_o < 0$ through $x_{s,1}$ -lengthening in stage 3, whilst $x_{s,2}$ and $x_{s,3}$ shortening on their respective transiting lines towards their concentric F–L curves when the myofibril is under eccentric contraction, or shortening on their (isometric) F–L curves when the myofibril is under isometric contraction. Because $\delta x_o = \frac{dx_o}{dy_o} \delta y_o$ with $\delta y_o < 0$, $\delta x_o > 0$ if and only if $\frac{dx_o}{dy_o} = \sum_{i=1}^3 \frac{1}{f'_s(x_{s,i}, p_{s,i})} < 0$. This verifies (r2) and in particular, for eccentric contraction, $f'_s(x_{s,i}, p_{s,i}) = k_{c,i}$ for $i = 2, 3$, and $f'_s(x_{s,1}, p_{s,1}) = a_{4,1} + p_{1,1}p_{2,1}e^{p_{2,1}\bar{x}_{p,1}}$ achieves the minimum $a_{4,1} + p_{1,1}p_{2,1}$ at $x_{s,1} = x_{p,1}$. At

the end of this stage of x_o -lengthening, x_o has reached $x_{o,3} = x_{o,2} + \delta x_{s,1} + \delta x_{s,2} + \delta x_{s,3}$ with $\delta x_{s,1} = x_{v,1} - x_{3,1}$ and, for $i = 2, 3$, $\delta x_{s,i} = (\hat{y}_{v,1} - \hat{y}_{2,1})/k_{c,i}$ (transiting from eccentric towards concentric curve) or $\delta x_{s,i} = f^{-1}(y_{v,1} - y_{2,1}, q_{s,i})$ (on the isometric curves). (r2) is verified for $x_{s,1}$ -lengthening in stage 3, whilst (r1) and (r3) are not invoked.

From $x_{o,3}$, further infinitesimally x_o -lengthening must be through $x_{s,1}$ lengthening in stage 4, whilst $x_{s,2}$ and $x_{s,3}$ lengthening on their transiting lines back towards their eccentric F-L curves in stage 1 when the myofibril is under eccentric contraction, or on their isometric F-L curves otherwise. This is always possible because, for $i = 1, 2, 3$, $f'_s(x_{s,i}, p_{s,i}) > 0$ and is continuous within stages 1 and 4. Hence, for any infinitesimal $\delta y_o > 0$, $\delta x_o = \sum_{i=1}^3 \delta x_{s,i}$ with $\delta x_{s,i} = f_s^{-1}(\delta y_o, p_{s,i})$ for $i = 1, 2, 3$ being infinitesimals. With arbitrarily many infinitesimal steps, $x_{s,i}$, for $i = 2, 3$, is back to $f^{-1}(\bar{y}_{2,1}, \bar{q}_{s,i})$, and $x_{s,2}$ further to $x_{2,2}$, and meanwhile $x_{s,1}$ reaches $f^{-1}(\bar{y}_{2,2}, \bar{q}_{s,1})$ in stage 4, and $x_{s,3}$ reaches $f^{-1}(\bar{y}_{2,2}, \bar{q}_{s,3})$ in stage 1. This is possible if and only if $\bar{y}_{2,2} \leq \bar{y}_{4,1}$ which is (r1) in the case of considering $x_{s,1}$ and $x_{s,2}$ only.

In a similar way of proving $x_{s,1}$'s traverse from stage 2 to stage 4, further infinitesimally x_o -lengthening will bring $x_{s,2}$ through stage 2 to stage 3 and then stage 4. Meanwhile, $x_{s,1}$ in stage 4 and $x_{s,3}$ in stage 1 will undergo stopping on their F-L curves, shortening and then lengthening on their transiting lines or their F-L curves (depending on the nature of the myofibril's contraction), and then lengthening on their F-L curves, until $x_{s,3}$ reaches stage 2. As in the case of $x_{s,1}$ -lengthening, (r1) and (r2) are required. In addition, if the myofibril is under isometric contraction, (r3) is also needed because, without the depth of $x_{s,1}$'s valley being lower than that of $x_{s,2}$, by x_o -lengthening, an increment in $x_{s,2}$ is not guaranteed infinitesimal, and so is the increment in x_o .

Any further x_o -lengthening will have to be lengthening $x_{s,3}$ throughout stages 2-4, and meanwhile $x_{s,1}$ and $x_{s,2}$ undergo stopping, shortening and lengthening in stage 4, which requires (r2) under eccentric or isometric contraction and (r3) under isometric contraction. Final infinitesimally x_o -lengthening to achieve it maximal must be through lengthening all the three sarcomeres in stage 4 without invoking (r1) to (r3).

Shortening x_o The details of the proof for shortening x_o under concentric or isometric contraction are very similar to those of lengthening x_o under eccentric or isometric contraction. Hence, for brevity, only the main steps of the proof for x_o -shortening are highlighted.

From the maximal length of x_o with $x_{s,i}$ for $i = 1, 2, 3$, being fully stretched, infinitesimally x_o -shortening has to be through shortening $x_{s,i}$ for $i = 1, 2, 3$ in stage 4. Recalling convention C2, once $x_{s,3}$ reaches $x_{v,3}$, further infinitesimally x_o -shortening has to be through $x_{s,3}$ -shortening in stage 3

and $x_{s,1}$ and $x_{s,2}$ lengthening on their transiting lines towards their eccentric F-L curves if the myofibril is under concentric contraction, or otherwise lengthening on their isometric F-L curves. These infinitesimal increments in x_o are possible if (r2) is satisfied, and $x_{s,3}$ can reach stage 2 from the right if and only if (r1) is true. If $x_{s,3}$ does not have a force valley, x_o -shortening would lead $x_{s,3}$ directly reaching stage 2 without invoking (r2), but (r1).

Further infinitesimally x_o -shortening has to be through $x_{s,3}$ -shortening in stage 2 and then stage 1, whilst $x_{s,1}$ and $x_{s,2}$ undergo stopping and then shortening on their transiting lines back to their concentric F-L curves if the myofibril is under concentric contraction, or otherwise shortening on their isometric F-L curves. This stage of x_o -shortening does not invoke (r1) to (r3) until $x_{s,2}$ reaches $x_{v,2}$ or directly reaches stage 2 if $x_{s,2}$ does not have a force valley.

In the general case of $x_{s,2}$ having a force valley, as in the passage of $x_{s,3}$ from stage 4 to stage 1, further infinitesimally x_o -shortening has to be through $x_{s,2}$ -shortening in stage 3 and $x_{s,3}$ and $x_{s,1}$ lengthening on their transiting lines towards their eccentric F-L curves if the myofibril is under concentric contraction, or otherwise lengthening on their isometric F-L curves. This stage of x_o -shortening until $x_{s,2}$ reaching stage 2 requires (r2), and in addition (r3) if the myofibril is under isometric contraction.

Without invoking (r1) to (r3), further infinitesimally x_o -shortening has to be through $x_{s,2}$ -shortening in stage 2 and then stage 1, whilst $x_{s,3}$ and $x_{s,1}$ shortening on their transiting lines back to their concentric F-L curves if the myofibril is under concentric contraction, or otherwise shortening on their isometric F-L curves, until $x_{s,1}$ reaching $x_{v,1}$. Thereafter, further infinitesimally x_o -shortening will cause $x_{s,1}$ shortening through stage 3 to stage 1, and meanwhile $x_{s,3}$ and $x_{s,2}$ undergo lengthening, stopping and shortening, which require (r2) and (r3). Final infinitesimally x_o -shortening is through all three sarcomeres shortening in stage 1 to reach their minimal lengths.

Proof of Corollary 1

Necessity of (r1) for this corollary is obvious from the proof of Proposition 1. Proof of sufficiency of (r1) focuses on examinations of effects of (r2) and/or (r3) failing since the proof is otherwise exactly the same as that of Proposition 1. In the cases of (r2) and/or (r3) failing, two types of discontinuity in the myofibril F-L relation, namely force and length jumps, can happen. In the former, for an infinitesimal δx_o there is a $\delta x_{s,i}$ which cannot be infinitesimal and hence the associated δy_o cannot be infinitesimal. In the latter, for an infinitesimal δy_o there is at least one $\delta x_{s,i}$ which cannot be infinitesimal and so is the associated increment δx_o .

Lengthening x_o Recall that up to $x_{o,2}$, x_o -lengthening has not invoked (r1) to (r3). Let therefore $(x_o, y_o) =$

$(x_{o,2}, \bar{y}_{2,1})$. Further x_o -lengthening requires $x_{s,1}$ -lengthening in stage 3. If (r2) fails, for an increment $\delta y_o \in (\bar{y}_{v,1} - \bar{y}_{2,1}, 0)$, $\frac{dy_o}{dx_o} > 0$ cannot produce an infinitesimal $\delta x_o > 0$ unless $\delta y_o < 0$ is not infinitesimal, a force jump in y_o .

Now, under (r1) and (r2), let $x_{s,1}$ be in stage 4, $x_{s,2}$ at the end of stage 2, and $x_{s,3}$ in stage 1. If (r3) fails, $x_{s,2}$ cannot reach $x_{v,2}$. This implies that to reach stage 4 for $x_{s,2}$, increment $\delta x_{s,2}$ cannot always be infinitesimal, which leads to a jump in x_o .

Similarly for $x_{s,3}$'s passage from stage 1 to stage 4, under (r1) but without (r2) and/or (r3), at least a force jump and/or a length jump will occur in x_o -lengthening.

Shortening x_o Similar arguments as in the case of x_o -lengthening apply to x_o -shortening, and hence the verification in this case is omitted.

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