HOSTED BY

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Journal of Sport and Health Science 7 (2018) 265–274

www.jshs.org.cn

Review

Basic science and clinical use of eccentric contractions: History and uncertainties

Kiisa C. Nishikawa [a], Stan L. Lindstedt [a,*], Paul C. LaStayo [b]

[a] *Center for Bioengineering Innovation and Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011, USA*
[b] *Department of Physical Therapy and Athletic Training, University of Utah, 520 Wakara Way, Salt Lake City, UT 86011, USA*

Received 6 December 2017; revised 30 December 2017; accepted 9 January 2018
Available online 20 June 2018

Abstract

The peculiar attributes of muscles that are stretched when active have been noted for nearly a century. Understandably, the focus of muscle physiology has been primarily on shortening and isometric contractions, as eloquently revealed by A.V. Hill and subsequently by his students. When the sliding filament theory was introduced by A.F. Huxley and H.E. Huxley, it was a relatively simple task to link Hill's mechanical observations to the actions of the cross bridges during these shortening and isometric contractions. In contrast, lengthening or eccentric contractions have remained somewhat enigmatic. Dismissed as necessarily causing muscle damage, eccentric contractions have been much more difficult to fit into the cross-bridge theory. The relatively recent discovery of the giant elastic sarcomeric filament titin has thrust a previously missing element into any discussion of muscle function, in particular during active stretch. Indeed, the unexpected contribution of giant elastic proteins to muscle contractile function is highlighted by recent discoveries that twitchin–actin interactions are responsible for the "catch" property of invertebrate muscle. In this review, we examine several current theories that have been proposed to account for the properties of muscle during eccentric contraction. We ask how well each of these explains existing data and how an elastic filament can be incorporated into the sliding filament model. Finally, we review the increasing body of evidence for the benefits of including eccentric contractions into a program of muscle rehabilitation and strengthening.

Keywords: Exercise; Force enhancement; Muscle mechanics; Rehabilitation; Titin/connectin

1. A Brief history of lengthening muscle contractions

As animals move through their environments, muscles must perform many functions to stabilize, propel, and decelerate their bodies. Muscles function not only as the source of work necessary for propulsion, but they are equally important in their function as brakes, converting kinetic energy of motion by recovering potential energy, or dissipating it as heat. For example, when moving downhill, gravity alone can result in sufficient kinetic energy that muscles must function as regulated brakes to decelerate the animal. Likewise, during running, because footfall always occurs before the center of mass moves over the foot, the first one-half of the stride necessarily stretches the hip and knee extensors. If the energy absorbed during this phase of the stride is recovered during the muscle shortening cycle, then work done by the muscle is enhanced.

Lengthening (eccentric) muscle contractions are distinguished by several unique properties. In 1924, Fenn[1] may have been the first to observe that force production requires much less energy if a muscle is stretched while active (and more energy if shortening, the so-called *Fenn effect*). Decades later, his mentor A.V. Hill remained sufficiently puzzled by this observation to speculate that stretched muscle may function as an adenosine triphosphate generator (see Lindstedt[2] for a discussion). Perhaps the difference in energy requirement between lengthening and shortening contractions was best demonstrated by Abbott et al.[3] using mechanically linked back-to-back stationary bikes. They showed that far less energy is required to resist than to propel the pedal movement.[4] Additionally, and linked to increased energy efficiency, maximum muscle force is much greater during eccentric contraction than during shortening contraction.[3]

Peer review under responsibility of Shanghai University of Sport.
* Corresponding author
E-mail address: stan.lindstedt@nau.edu (S.L. Lindstedt).

https://doi.org/10.1016/j.jshs.2018.06.002

2. Mechanisms of eccentric contraction

When active muscle is stretched, absorbing mechanical energy, there are 2 fates of that energy: it can be lost as heat or stored as elastic potential energy. This stored energy can increase the work done during subsequent muscle shortening while minimizing the energy cost. The stored energy is only available for a short time, which likely sets stride frequency during locomotion.[5] There has been considerable speculation as to how and where this energy is stored.[6–10] However, tendons outside the muscle and collagen within account for only a small fraction (~4%) of energy storage.[8,9] Thus the sarcomere itself must store most of the recoverable energy. There are apparently only 2 candidates within the sarcomere that could assume this function, the cross bridges and the giant elastic titin filaments.[11]

Given that nearly a century has passed since the high-force and low-energy cost of eccentric muscle contractions were first described by Fenn,[1] it is surprising that so little progress has been made in identifying the biophysical and biochemical basis for these muscle properties that play important roles in the biomechanics and control of movement.[12] Herein, we review the alternative hypotheses, attempt to understand why definitive answers have not been forthcoming, and suggest potentially fruitful experiments that could help to rule out alternative hypotheses. The history of the discovery of mechanisms underlying the "catch" phenomenon in muscles of invertebrates, which shares many features with eccentric contraction in vertebrate muscles, suggests some potential approaches.

3. Force enhancement during stretch of active muscle

Because the only active players in muscle sarcomeres were long thought to be acto–myosin cross bridges, until recently nearly all mechanistic theories of eccentric contraction attributed the increased force during and after stretch of active muscles to cross-bridge properties.[6,7,13,14] Reverse engineering—deducing the function of the cross bridges from the macroscopic behavior of muscles—is a valuable scientific tool for generating new hypotheses. Deductive reasoning, however, is less useful for hypothesis testing because it is prone to hidden and usually untested assumptions and can lead down a spurious path (e.g., Hill's adenosine triphosphate generation) when assumptions are false or important facts are missing. Although the goal of muscle physiology should instead be to predict the macroscopic behavior of muscle from an understanding of the properties of its component parts,[15] the practicality of a more inductive approach is limited by the technical challenges of measuring cross-bridge properties directly. Yet if we are necessarily constrained to using a deductive approach, it is all the more important to acknowledge its limitations.

Force enhancement in muscles during and after active stretching is a classic example of deductive reasoning. The standard and nearly universal approach has been to measure the macroscopic properties of stretched muscle and infer the properties of the cross bridges directly from these measurements,[6,7] explicitly or implicitly assuming that the cross bridges alone are responsible for producing the macroscopic properties. Despite the evident circularity of this reasoning, it has become surprisingly difficult even to suggest that there is room for alternative mechanisms.

Although there is no fundamental theoretical problem with cross bridges storing energy during stretch, their small size, short duration of attachment, and rapid detachment from actin[15] impose significant constraints on their ability to store energy. To explain the lower energy cost of eccentric contractions, cross-bridge models require ad hoc assumptions. Untestable assumptions regarding cross-bridge properties, such as stiffness, duty ratio, and energy states, are therefore required for estimating the potential of cross bridges to store energy during stretch. Early work assumed that all of the instantaneous elasticity of muscle resides in the cross bridges[13] and that the cross bridges alone account for all of the increased force during stretch.[6,7] Yet, by 2003, the estimated contribution of cross bridges to energy storage during active muscle stretch was only 12%, with cross-bridge elasticity accounting for a mere 2% of the energy.[9] To understand this change in the perceived contribution of cross bridges to active stretch, it is instructive to examine this history in greater detail.

Assuming that "there is a virtually instantaneous elasticity within each cross bridge", Huxley and Simmons[13] concluded "we now believe that the instantaneous elasticity (or at least the greater part of it) resides in the cross bridges themselves". Lombardi and Piazzesi[6] made careful measurements showing that the force during active lengthening of isolated frog muscle fibers was nearly double the isometric force. On the basis of these experiments, they concluded that "steady lengthening of muscle fibers induces a cross-bridge cycle characterized by fast detachment of cross bridges extended beyond a critical level". Their mathematical model suggested that "reattachment of forcibly attached cross bridges is 200 times faster than attachment of cross bridges which detach after completion of the cycle". This deductive model was developed further by Piazzesi and Lombardi.[7]

Two different lines of evidence contributed to the changing view of cross bridges between 1995 and 2003. The first was the observation from molecular motors that the duty ratio of myosin II in muscle sarcomeres must necessarily be low, likely <20% and possibly much lower, because the distance between successive binding sites on actin (~36 nm) is too far to be traversed within a single cross-bridge cycle.[16] The conclusion, now generally accepted,[17] is that the number of cross bridges attached at any given time (~20%) is only a small fraction of the value (77%) typically assumed in previous studies[6,7] and rationalized on the basis of X-ray diffraction and other empirical observations. The debate is nicely captured in Huxley's letter to the editors and Howard's reply.[18]

The second line of evidence was the observation that the compliance of the thin[19] and thick filaments[20] also contributes significantly (~70%) to muscle compliance, so that muscle stiffness is not directly proportional to the number of attached cross bridges.[16,18] Recent studies have quantified the force-dependent structural changes in thick and thin filaments that occur on stretch of passive and active muscle,[21–23]

demonstrating an intriguing correlation between these structural rearrangements and force during stretch. However, the deduction that the cross bridges are responsible for the observed structural rearrangements and that the rearrangements *per se* allow more cross bridges to form require further experiments to demonstrate causation.

Linari et al.[9] concluded that the thick and thin filaments contribute ~4% and the cross bridges contribute only ~12% to energy stored during active stretch (with 2% attributed to cross-bridge elasticity and 10% to redistribution of cross bridges to different energy states). Although Linari et al.[9] point out that the assumptions made by Lombardi and Piazzesi[6] were entirely reasonable at the time, the point we make here is that until quantities such as cross-bridge duty ratio, stiffness, and force can be measured directly, estimates of cross-bridge contributions to force enhancement based on deductive reasoning should be regarded with due skepticism. Yet, despite significant revisions to the cross-bridge theory in the late 1990s, the following views are still widely held by most muscle physiologists: (1) cross bridges not only can, but actually do, account for all of the energy stored during stretch of active muscle and (2) muscle stiffness is proportional to the number of attached cross bridges. Until technological developments enable direct measurements of cross-bridge properties, it would be wise to insist on an explicit statement of assumptions and to view conclusions based on deductive reasoning as speculative.

In their detailed analysis of energy storage during stretch of active muscle fibers, Linari et al.[9] estimated that tendon (~1.5%), thick and thin filaments (~4.0%), cross bridges (~12%), and titin (~15.5%) together explained only ~34% of the total energy stored in muscle during active stretch. Their estimates of the contribution of titin were based on a model in which sarcomere inhomogeneity increases passive titin force by stretching 4.5% of sarcomeres to an average sarcomere length of 3.5 μm, and that the remaining 95.5% of sarcomeres remain at the same length. The fact that ~66% of the energy stored in muscle during active stretch remained unexplained makes it apparent that additional mechanisms remain to be discovered.

4. Residual force enhancement after stretch of active muscle

Numerous theories have been suggested to explain the long-lasting increase in force that persists after stretching active muscle, or residual force enhancement.[11,24] The theories basically fall into 3 categories: cross-bridge theories, sarcomere length inhomogeneity theories, and theories based on engagement of passive elements, now thought to be titin.[11] Minozzo and Lira[11] suggested that none of these theories can be conclusively ruled out and, furthermore, that all 3 mechanisms could coexist and are therefore not mutually exclusive.

4.1. Increased force of cross bridges

In contrast with storage of energy during stretch, the relatively fast cycling of the cross bridges on and off of the thin filaments creates a fundamental theoretical problem for explaining residual force enhancement. The main problem is that cross bridges are too small (~5.5 nm with a working stroke of 12−18 nm),[9] remain attached to actin for only a short time,[15] and detach at too high a frequency to explain the residual force enhancement that persists after stretch over long distances of hundreds of nanometers and for seconds or even minutes. Using a Huxley 2-state cross-bridge model, Harry et al.[25] could find no reasonable value of cross-bridge strain that could account for residual force enhancement. Walcott and Herzog[26] also showed that standard cross-bridge models cannot account for residual force enhancement without requiring *ad hoc* assumptions.

Despite the fundamental difficulties associated with their small strain and rapid cycling, a variety of cross-bridge mechanisms has nevertheless been proposed to account for residual force enhancement. These mechanisms include myosin light chain phosphorylation,[27] changes in myofilament lattice spacing observed during stretch using X-ray diffraction,[28] and increased cross-bridge force.[29] However, neither an increase in the force per cross bridge nor an increase in the number of attached cross bridges alone can account for the long duration of residual force enhancement, which is difficult to reconcile with the cross-bridge cycling that must necessarily dissipate stored energy.

4.2. Sarcomere length nonuniformity

In fact, it was the inability of cross-bridge mechanisms to account for residual force enhancement[25] that led to the development of the sarcomere length nonuniformity theory.[30,31] This theory originally stated that "lengthening of muscle on the descending limb (and probably plateau) of the length−tension curve takes place by extremely rapid, uncontrolled lengthening of sarcomeres, or half-sarcomeres, in order from weakest to strongest, with only very slow lengthening of the others. The fast velocity necessarily arises from the fact that the weakest sarcomeres will lengthen more rapidly than the others and, in the process, become even weaker."[31]

Notwithstanding numerous observations demonstrating that sarcomere length does vary in muscle fibers and myofibrils,[32−34] the sarcomere length nonuniformity theory makes several predictions that have been shown repeatedly to be false: (1) that sarcomere length variability must be greater after stretch than during isometric contraction—it is not; (2) that force enhancement must be restricted to the plateau and descending limb of the force−length relationship—it is not; and (3) that force after stretch must not exceed the maximum isometric force—which it does.[35,36] When the length of every sarcomere in a series was measured in single myofibrils, the distribution of sarcomere lengths was more uniform in the force-enhanced state after stretch than after isometric contractions at the stretched length.[34] Force enhancement, although small, occurs on the ascending limb of the force−length relationship.[35−37] Finally, the force after active stretch may exceed the isometric force at the stretched length.[35,36] Additionally, force enhancement has been observed in single

sarcomeres and myofibrils in the absence of sarcomere length nonuniformity, refuting the necessity of length nonuniformity for the development of residual force enhancement.[33,38]

The length nonuniformities are also observed on active stretch of a single half sarcomere.[39,40] However, the magnitude and duration of the observed increases in force owing to half-sarcomere length nonuniformities are not large enough to account for residual force enhancement,[35,41,42] in addition to suffering from many of the same limitations as earlier versions of the theory.[43] The persistence of this theory despite the disproof of its central hypotheses is a strong demonstration that the field is in need of alternative hypotheses.

5. Titin, a giant elastic filament in muscle sarcomeres

There is increasing evidence that cross bridges, the engines of muscles, must have an elastic partner to account for the observed properties of eccentric contraction. Given the apparent inability of cross bridges alone or sarcomere length homogeneity to account for force enhancement, the likelihood that titin plays a role in eccentric muscle contraction is a promising alternative hypothesis. Because the cross bridges are small and their maximum extension is short, they likely have an elastic partner that can store energy when stretched over long distances.

Interestingly, the same year the sliding filament hypothesis was presented, Huxley and Hanson[44] speculated that myofibrils must possess an elastic element that is essential for maintaining the position of thick and thin filaments within muscle sarcomeres. They suggested that this undiscovered elastic filament should run from Z-disk to Z-disk, spanning the length of the sarcomere. They even named this unseen but necessary filament the S filament for stretch[45] (see reviews[46,47]). By the time that this hypothetical superthin third filament was identified via electron microscopy,[48] this idea too was met with skepticism even by its original proponents.[46,49] By then, it seemed that all observed properties of muscle could be attributed to the cross bridges, and thus the third filament was deemed irrelevant. For more details of this controversy, see discussions by Rall[46] and Lindstedt and Nishikawa.[50]

Electron microscopic evidence for a superthin sarcomeric filament[48,51] existed long before the protein, originally named connectin, was identified.[52] Three years later, Wang et al.[53] described an enormous sarcomeric protein that, as the largest known protein, was called *titin*. It was subsequently confirmed that connectin and titin were the same protein; [54] but, contrary to normal priority, the name titin remains more commonly used. Fürst et al.[55] were the first to demonstrate, using anti-titin antibodies, that the giant titin molecule extends continuously from the Z-disk to the M-line of striated muscle sarcomeres. Since its discovery and description, titin has been recognized as just one of a ubiquitous large family of giant sarcomeric proteins, including twitchin and sallimus, that are similar structurally and have been found recently to regulate aspects of force generation and maintenance.[56] These proteins include similar ones found in virtually all metazoans except jellyfish.[50]

While the structure of titin was being deciphered, so too were its functions. One of those was as a scaffold on which the sarcomere was built.[57] It was a long road of discovery before the elastic nature of this giant protein was characterized by Labeit and Kolmerer,[58] who described the 2 key I-band regions of this molecule, a tandem Ig domain closer to the Z-disk and a PEVK region—named for its most common amino acids, namely, proline (P), glutamate (E), valine (V), and lysine (K)—closer to the thick filament, which differ greatly in their elasticity. When titin is stretched passively, the more compliant tandem Ig region extends with low force whereas the stiff PEVK region requires much more force to extend.[59–62]

6. Titin's role in active muscle

Upon discovery of the titin protein, researchers naturally sought a role for titin in active muscle contraction.[63] Early work by Horowits and colleagues[64,65] demonstrated that titin prevents axial misalignment of thick filaments during active muscle contraction, which enables the development of high isometric forces.[66] It is also becoming increasingly accepted that titin plays a role in length-dependent activation, not only in the Frank–Starling mechanism of the heart,[22] but also in vertebrate skeletal muscle.[67–69] Although the mechanism for titin's role in length-dependent activation was initially thought to involve radial forces that moved the cross bridges closer to the thin filaments, several studies have now demonstrated that increasing activation depends on titin stiffness rather than lattice spacing.[22,67] It has been suggested that structural changes in thick and thin filaments, mediated by titin, may contribute indirectly to the length dependence of activation by modulating thin filament activation or cross-bridge kinetics.[66] Although the purely passive stiffness of cardiac titin isoforms may be sufficient to produce strain in the thick and thin filaments, the passive stiffness of titin in skeletal muscle seems to be insufficient to support a role in the axial alignment or rearrangement of the thick and thin filaments. For titin to play a role in the axial alignment or structural rearrangement of the relatively much stiffer thick and thin filaments in skeletal muscle, an activation-dependent increase in titin stiffness is required.

7. A role for titin in eccentric contraction

On observing that single muscle fibers shorten faster in the force-enhanced state, Edman et al.[70] were the first to suggest that force enhancement might involve recruitment of viscoelastic elements. Edman and Tsuchiya[71] reached a similar conclusion using load–clamp and unloaded shortening tests. Herzog and Leonard[72] further demonstrated that, when the cat soleus muscle was stretched, the enhanced force persisted for several seconds after active stretch, even after deactivation of the stretched muscle. The increased passive tension that persists after deactivation accounts for some of the force enhancement after active stretch, the simplest interpretation being that a structural element, now thought to be titin, must contribute to force enhancement.[73] Likewise, when muscle fibers are

stretched, their static tension increases during the early stages of muscle activation.[74–77]

Increasing evidence suggests that titin stiffness increases with Ca^{2+} influx in muscle sarcomeres. Leonard and Herzog[78] demonstrated that, if single myofibrils are activated by Ca^{2+} at a sarcomere length of 2.4 μm and stretched to a length beyond the thick and thin filament overlap (sarcomere length >3.8 μm), the force of myofibrils increases more rapidly with stretch than it does in passive myofibrils.[79] Furthermore, when sarcomeres are stretched beyond overlap, it is impossible for cross bridges *per se* to contribute directly to active force. Finally, no decreased tension with stretch was observed when the myofibrils were stretched slowly to long sarcomere lengths, implying little or no unfolding of Ig domains.[11,80] These observations taken together led Leonard and Herzog[78] to speculate that titin may bind to actin when Ca^{2+} is present, decreasing titin's free length and increasing its stiffness,[81] in addition to relatively small direct effects of Ca^{2+} on titin stiffness.[73,82]

8. Alternative hypotheses for titin's role in eccentric contraction

Early studies demonstrated that titin fragments decreased the motility of actin filaments on myosin in a calcium-dependent fashion,[83] suggesting a potential role for titin–actin interactions in active muscle contraction. The demonstration that titin stiffness increases upon calcium activation of muscle[78,79] also suggested several alternative hypotheses that N2A or PEVK titin might bind to actin in active muscle, thereby shortening and stiffening the titin spring, which could account for the greater forces and lesser energy costs of eccentric contractions.[78,79,84–87] Rode et al.[84] suggested that titin binds to actin at the same sites as myosin, exposed during calcium activation of the thin filaments, resulting in enhanced force during stretch and competitive inhibition of force during shortening. Schappacher-Tilp et al.[87] developed a model based primarily on data from Leonard and Herzog's[78] experiments in which PEVK titin binds to actin only during active stretching and not during isometric contraction, perhaps to suggest that binding sites on titin are exposed only during stretching. The site of titin–actin binding was a free variable, initially set to the most proximal PEVK residue. Their model is similar to that from Nishikawa et al.,[85] who hypothesized that titin's N2A region would be ideal for modulating titin stiffness in active muscle owing to its location at the border between Ig domains that elongate at low stiffness and the much stiffer PEVK region. That N2A titin might bind to actin is also suggested by deficits in titin activation[86] and force enhancement[88] in muscles from myositis with muscular dystrophy (mdm) mice, which carry a deletion in N2A titin.[85]

Despite the apparent usefulness of titin–actin interactions in explaining muscle properties during eccentric contraction, a number of cosedimentation and *in vitro* motility studies using recombinant titin fragments have failed to find evidence for interactions between titin and actin in skeletal muscle,[89,90] whereas several studies have demonstrated that calcium decreases the strength of interactions between PEVK titin and actin in cardiac muscle.[91,92] Yet, an absence of evidence is not evidence of absence, especially in the case of a giant protein composed of tens of thousands of amino acids. In fact, a careful analysis shows that no previous investigations of titin–actin interactions have included the N2A–PEVK border region (amino acids 5508–5618 in human soleus titin X90569,[58] which includes 53 of the 83 amino acids deleted in mdm).[93] Thus it remains possible that the distal N2A region and the proximal PEVK region interact with actin in active skeletal muscle.

In an analysis of Leonard and Herzog's[78] experiments on myofibrils, Granzier[80] suggested that, although difficult technically, stretching active and passive myofibrils labeled with fluorescent titin antibodies could potentially be used to test the hypothesis that interactions with actin decrease or prevent the elongation of titin upon activation, compared with purely passive elongation. In fact, DuVall et al.[94] demonstrated recently, using an F146 antibody that binds to titin near the A-band, that elongation of titin segments changes on activation in a manner consistent with a calcium-dependent, but not an actin-dependent, increase in titin stiffness. To definitively rule out a role for titin–actin interactions in eccentric muscle contraction will require investigations of the N2A–PEVK border region, as well as experiments using additional antibodies that bind to titin in the I-band.

9. Hypothesis testing, elastic filaments, and a lesson from the mechanism of invertebrate catch

Known as early as 1885,[95] the catch phenomenon in muscles of invertebrates shares with eccentric contraction the properties of resistance to stretch and maintenance of force at low energetic cost.[96] Catch is now generally accepted as resulting from binding of dephosphorylated twitchin, a titin ortholog,[97] to actin. The history of hypothesis testing and eventual elucidation of the catch mechanism[95] provides a cautionary tale for theories of eccentric contraction and may suggest experimental approaches that could help to resolve the mechanisms.

In molluscan catch, an elastic element develops upon muscle activation, persists for long periods after deactivation, and adjusts its stiffness during shortening to maintain its force at a shorter length.[98] Before the discovery and general acceptance of the twitchin-based mechanism, many of the same cross-bridge theories proposed to explain residual force enhancement were also proposed as mechanisms of catch, including cross-bridge mechanisms[99] and phosphorylation of the myosin light chain.[100] A role for the giant twitchin protein was unexpected.[95] Until Butler and Siegman[101] observed that only the phosphorylation state of twitchin was correlated with the catch state, nobody had even suggested that twitchin might play a role. Although no fewer than 26 proteins are phosphorylated in the catch state, Yamada et al.[56] demonstrated that catch could be observed in an *in vitro* assay containing only myosin, actin, and twitchin, and that catch depended only on the phosphorylation state of twitchin. Yet, despite this demonstration, the idea that catch was due to cross bridges persisted.[102,103] Later studies demonstrated that Ca^{2+} influx triggers dephosphorylation of twitchin and that binding of dephosphorylated

twitchin to actin is sufficient to explain catch,[104,105] although the question remains as to whether twitchin binding to actin is the only mechanism.[95] By analogy, the history of investigations into the mechanism of catch suggests that a definitive explanation of the mechanisms of force enhancement awaits demonstration of the necessity of the critical elements in an *in vitro* system, as well as biochemical and biophysical evidence supporting the sufficiency of the mechanism to explain the phenomenon of force enhancement. Even with these demonstrations, additional mechanisms could contribute as well.

These arguments demonstrate that our understanding of the mechanistic basis of eccentric contraction is surprisingly incomplete after nearly 100 years of investigation. The history of inquiry into the mechanisms demonstrates the vulnerability of muscle physiology as a fundamentally deductive science to underlying assumptions regarding cross-bridge properties and also to the unexpected alternative hypothesis that giant proteins likely play a role. The example of invertebrate catch demonstrates that giant sarcomeric proteins are important players in regulating the contractile state of animal muscles. If, as is generally believed,[66] titin is to play a role in stabilizing the axial position of thick filaments in the sarcomere and in the structural rearrangement of thick and thin filaments in length-dependent activation, then titin stiffness must increase substantially upon muscle activation, as observed by Leonard and Herzog[78] and Powers et al.[79] At this point in time, it seems increasingly likely that titin plays a major role in eccentric muscle contraction, although many of the molecular mechanisms remain to be discovered. The way forward will be technically challenging, but it seems likely that experiments to definitively rule out alternative hypotheses will be forthcoming in the next decade or two.

10. Eccentric contractions, muscle damage, and exercise interventions

Just as the mechanisms of eccentric contractions have remained controversial, so too have the benefits of lengthening contractions as a clinical tool.[106] The parallel histories of the basic understanding of eccentric contractions and their clinical usefulness is sufficiently striking that we conclude this review with a brief discussion of eccentric exercise interventions.

Lengthening, or eccentric, contractions have been strongly associated with muscle soreness and impairment of muscle function. Not surprisingly, this correlation was interpreted as a causal relationship. Thus, in 1984, Edwards and colleagues[107] summarized, "Recent physiological studies have shown that eccentric contractions produce considerable muscle damage in normal healthy subjects." They even recommended that therapeutic interventions intended for muscle strengthening "may need to be altered to avoid eccentric contractions". It was soon documented that eccentric contractions were indeed associated with ultrastructural damage to the sarcomeres,[108] providing strength to this apparent causal relationship. In addition, it was also conjectured that eccentric damage resulted in muscle restructuring. Thus a second correlative property was added to the list: an initial bout of damaging, especially eccentric, exercise was a necessary precursor to initiate muscle hypertrophy. Although there was no evidence that either of these postulates is true, these apparently invariant properties became entrenched dogma. In fact, eccentric exercise need not cause any muscle damage nor is damage a necessary precursor for muscle growth.[109]

Certainly, high eccentric forces in muscles naïve to eccentric contractions, or in muscles accustomed only to low forces, can produce damage. However, if the magnitude and duration of the eccentric forces are increased gradually over time (1−3 weeks) in repeated bouts, an effect with unclear mechanisms,[110] no damage, inflammation, or soreness occurs.[109,111,112] Despite this evidence, high levels of skepticism regarding the clinical adoption of eccentric exercise in rehabilitation endured. More evidence was needed to establish the overload principle,[113] the notion that inducing high eccentric forces is a suitable stimulus for muscle growth and increasing strength. Several studies[109,114,115] ultimately debunked the myth that damage after eccentric muscle activity is obligatory, thus establishing the potential for eccentric resistance exercise in rehabilitation. With the possible exception of rehabilitation for chronic tendinopathies, where a pain response to eccentric exercise is promoted and required for efficacious treatment,[116] eccentric dosing in rehabilitation is founded on a no-injury response.

A key concept when applying nondamaging, eccentric activity into a rehabilitation exercise framework is that the loading dosage must integrate the protective effect of repeated exposures with progressively increasing loads over several sessions.[117,118] Judicious eccentric dosing that ultimately capitalizes on high-force production at a low-cost requirement can be applied to muscle training and enhance physical functioning in frail or otherwise exercise-limited individuals who may lack the energy to sufficiently load their muscles without assistance. Attending to muscle weakness and atrophy (without injury) in these patient populations is a critical objective in rehabilitation. If not reversed, these muscle impairments precipitate a downward spiral of greater muscle wasting and weakness and further exacerbate physical dysfunction. In some cases, it can also increase the risk of a life-threatening fall.

The early studies with healthy subjects[114,115] demonstrated the rehabilitation potential of high-force, low-cost eccentric resistance exercise; moderate metabolic loads achieved with concentric exercise on an ergometer at 100 W could be reproduced with 400−500 W of eccentric exercise on a specialized eccentric ergometer without undue soreness or damage. The suite of clinical studies with patient populations that followed helped to highlight that eccentric exercise as a safe, feasible, and efficacious supplement to resistance exercise for rehabilitation purposes. For example, older adults with minimal left ventricular dysfunction and no exertional ischemia exercising eccentrically produced 4-fold greater muscular stress and improvements in the distance walked in 6 min without overstressing the cardiovascular system, that is, at cardiovascular and metabolic levels similar to those observed during concentric exercise.[119,120] Similarly, those with severe chronic airway obstruction (e.g., forced expiratory volume <50% of the predicted value) achieved nondamaging, high negative work levels during eccentric ergometry exercise with tolerable levels of leg

fatigue and dyspnea compared with concentric ergometry exercise.[121]

Additional studies support the decreased metabolic strain of eccentric exercise[122] and the application of eccentric exercise protocols in patients with cardiac conditions[120,123–126] because improvements in muscle and physical functioning are equivalent to outcomes after standard concentric modes of exercise. The eccentric outcomes, however, can occur at a lower metabolic cost.[117] Furthermore, studies with diverse patient populations—those living with chronic diseases (cancer,[127] progressive neurologic conditions,[128] or chronic obstructive pulmonary disease[121]), or after anterior cruciate ligament reconstruction,[129,130] total knee arthroplasty,[131] or a knee surgical procedure resulting in atrophy, weakness, fatigue, and mobility deficits—demonstrate a reversal of many of these impairments over a course of 6−16 weeks of eccentric exercise. Analogous eccentric outcomes in postmenopausal women[132] and adults with type 2 diabetes[133] have also been linked with positive insulin and glycemic control responses.

The early "doctrine" espousing eccentrics as dangerous and having no clinical usefulness has now been replaced. The current and collective data regarding eccentric resistance exercise advocate for its use as evidenced by the fact that eccentrics are now incorporated into clinical guidelines after anterior cruciate ligament reconstruction[134] and by editorial commentaries advocating the use of eccentrics for mitigating muscle and functional deficits in older adults.[135] A hallmark clinical study[112] with older (mean age, 80 years), frail, female and male patients helped to catalyze the rehabilitation potential of eccentric exercise. It became clear that 10−20 min of eccentric resistance exercise 3 times per week over 11 weeks can occur without injury, even in a frail population, and that the resultant muscle strength and size increases parallel a decrease in fall risk. Despite the small sample size, these effects occurred to a greater extent compared with those older frail adults who performed traditional resistance exercises. Collectively, these eccentric studies with patient populations repeatedly demonstrate a tolerance of progressive eccentric loading with increases in negative work from 2-fold to 10-fold. Moreover, the perceived exertion to perform these eccentric exercises never exceeds a somewhat hard level. Thus adults and especially older adults seem to be more willing to adhere (>90%) to the exercise. Finally, a recent, large, clinical trial with older patients who have fallen[136] reinforced the notion that eccentric training can be successfully implemented in fall prevention efforts. In older adults (>75 years of age) with comorbid disease conditions (>5) who experienced a fall within the past year, a decrease in fall events occurred over a 9-month period after eccentric training, although the comparable at-risk older population experienced equivalent fall prevention benefits from traditional resistance exercise.

11. Eccentric contraction and the science of discovery

Science is most rewarding when unifying principles emerge. For that reason, scientists tend to cling to ideas, perhaps inadvertently establishing doctrine based on marginal evidence. Thus novel concepts, if at odds with the accepted dogma, are initially rejected, even though the supporting evidence may be far greater than the evidence that was required initially to establish that dogma. Experimental evidence is interpreted through the lens of the currently accepted paradigm. Because science is conservative and innately resistant to innovation, progress at times is unreasonably deliberate. The more radical or transformative the departure from the accepted scientific norm, the greater the resistance to its acceptance. A wonderful example is Eldridge and Gould's hypothesis[137] that evolution occurs via punctuated equilibria rather than the established dogma of phyletic gradualism. In the accompanying editorial introduction,[137] Schopf writes, "Throughout their essay, however, runs a larger and more important lesson: *a priori* theorems often determine the results of 'empirical' studies, before a shred of evidence is collected. This idea, that theory dictates what one sees, cannot be stated too strongly." There is sufficient scientific inertia that new ideas must overcome an enormous activation energy that was virtually never required of the evidence used to establish the paradigm. The challenges of directly observing cross bridges provide ideal conditions for attributing their properties, deduced from muscle behavior, as accountable for eccentric contraction.

Acknowledgments

This work was supported by Kiisa Nishikawa: the National Science Foundation (IOS-0732949, IOS-1025806, and IOS-1456868 IIP-1237878 and IIP-1521231), the W.M. Keck Foundation, and the Technology Research Initiative Fund of Northern Arizona University and Paul LaStayo: National Institutes of Health (R01AG031255; R21CA114523; R21AG18701 (with Stan Lindstedt)), the Foundation for Physical Therapy, and the University of Utah Research Foundation.

Authors' contributions

KCN, SLL, and PCL all participated in the drafting, writing, and revising of the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Competing interests

KCN has no competing interests. SLL and PCL are co-inventors on an eccentric ergometer licensed to BTE Technologies, Inc., Hanover, MD, USA. Neither SLL nor PCL has received any financial incentives (e.g., reimbursements, fees, royalties, funding, or salary) from the company or stemming from the contents of this manuscript or any related published papers.

References

1. Fenn WO. The relation between the work performed and the energy liberated in muscular contraction. *J Physiol* 1924;**58**:373–95.
2. Lindstedt SL. Skeletal muscle tissue in movement and health: positives and negatives. *J Exp Biol* 2016;**219**:183–8.

3. Abbott BC, Bigland B, Ritchie JM. The physiological cost of negative work. *J Physiol* 1952;**117**:380–90.
4. Elmer SJ, LaStayo PC. Revisiting the positive aspects of negative work. *J Exp Biol* 2014;**217**:2434–6.
5. Schaeffer PJ, Lindstedt SL. How animals move: comparative lessons on animal locomotion. *Compr Physiol* 2013;**3**:289–314.
6. Lombardi V, Piazzesi G. The contractile response during steady lengthening of stimulated frog muscle fibres. *J Physiol* 1990;**431**:141–71.
7. Piazzesi G, Lombardi V. A cross-bridge model that is able to explain mechanical and energetic properties of shortening muscle. *Biophys J* 1995;**68**:1966–79.
8. Linari M, Piazzesi G, Dobbie I, Koubassova N, Reconditi M, Narayanan T, et al. Interference fine structure and sarcomere length dependence of the axial x-ray pattern from active single muscle fibers. *Proc Natl Acad Sci U S A* 2000;**97**:7226–31.
9. Linari M, Woledge RC, Curtin NA. Energy storage during stretch of active single fibres from frog skeletal muscle. *J Physiol* 2003;**548**:461–74.
10. Pinniger GJ, Ranatunga KW, Offer GW. Crossbridge and non-cross-bridge contributions to tension in lengthening rat muscle: force-induced reversal of the power stroke. *J Physiol* 2006;**573**:627–43.
11. Minozzo FC, Lira CA. Muscle residual force enhancement: a brief review. *Clinics* 2013;**68**:269–74.
12. Nishikawa KC, Monroy JA, Powers KL, Gilmore LA, Uyeno TA, Lindstedt SL. A molecular basis for intrinsic muscle properties: implications for motor control. *Adv Exp Med Biol* 2013;**782**:111–25.
13. Huxley AF, Simmons RM. Proposed mechanism of force generation in striated muscle. *Nature* 1971;**233**:533–8.
14. Sugi H. Tension changes during and after stretch in frog muscle fibres. *J Physiol* 1972;**225**:237–53.
15. Marcucci L, Yanagida T. From single molecule fluctuations to muscle contraction: a Brownian model of A.F. Huxley's hypotheses. *PLoS One* 2012;**7**:e40042. doi:10.1371/journal.pone.0040042.
16. Howard J. Molecular motors: structural adaptations to cellular functions. *Nature* 1997;**389**:561–7.
17. Fusi L, Percario V, Brunello E, Caremani M, Bianco P, Powers JD, et al. Minimum number of myosin motors accounting for shortening velocity under zero load in skeletal muscle. *J Physiol* 2017;**595**:1127–42.
18. Huxley A. How molecular motors work in muscle. *Nature* 1998;**391**:239–40.
19. Wakabayashi K, Sugimoto Y, Tanaka H, Ueno Y, Takezawa Y, Amemiya Y. X-ray diffraction evidence for the extensibility of actin and myosin filaments during muscle contraction. *Biophys J* 1994;**67**:2422–35.
20. Dobbie I, Linari M, Piazzesi G, Reconditi M, Koubassova N, Ferenczi MA, et al. Elastic bending and active tilting of myosin heads during muscle contraction. *Nature* 1998;**396**:383–7.
21. Linari M, Brunello E, Reconditi M, Fusi L, Caremani M, Narayanan T, et al. Force generation by skeletal muscle is controlled by mechanosensing in myosin filaments. *Nature* 2015;**528**:276–9.
22. Ait-Mou Y, Hsu K, Farman GP, Kumar M, Greaser ML, Irving TC, et al. Titin strain contributes to the Frank-Starling law of the heart by structural rearrangements of both thin- and thick-filament proteins. *Proc Natl Acad Sci U S A* 2016;**113**:2306–11.
23. Fusi L, Brunello E, Yan Z, Irving M. Thick filament mechano-sensing is a calcium-independent regulatory mechanism in skeletal muscle. *Nat Commun* 2016;**7**:13281.
24. Edman KA, Elzinga G, Noble MI. Residual force enhancement after stretch of contracting frog single muscle fibers. *J Gen Physiol* 1982;**80**:769–84.
25. Harry JD, Ward AW, Heglund NC, Morgan DL, McMahon TA. Cross-bridge cycling theories cannot explain high-speed lengthening behavior in frog muscle. *Biophys J* 1990;**57**:201–8.
26. Walcott S, Herzog W. Modeling residual force enhancement with generic cross-bridge models. *Math Biosci* 2008;**216**:172–86.
27. Edman KA. The force bearing capacity of frog muscle fibres during stretch: its relation to sarcomere length and fibre width. *J Physiol* 1999;**519**:515–26.
28. Amemiya Y, Iwamoto H, Kobayashi T, Sugi H, Tanaka H, Wakabayashi K. Time-resolved X-ray diffraction studies on the effect of slow length changes on tetanized frog skeletal muscle. *J Physiol* 1988;**407**:231–41.
29. Mehta A, Herzog W. Cross-bridge induced force enhancement. *J Biomech* 2008;**41**:1611–5.
30. Morgan DL. New insights into the behavior of muscle during active lengthening. *Biophys J* 1990;**57**:209–21.
31. Morgan DL. An explanation for residual increased tension in striated muscle after stretch during contraction. *Exp Physiol* 1994;**79**:831–8.
32. Julian FJ, Morgan DL. The effect on tension of non-uniform distribution of length changes applied to frog muscle fibres. *J Physiol* 1979;**293**:379–92.
33. Johnston K, Jinha A, Herzog W. The role of sarcomere length non-uniformities in residual force enhancement of skeletal muscle myofibrils. *R Soc Open Sci* 2016;**3**: 150657. doi:10.1098/rsos.150657NID!12345.
34. Joumaa V, Leonard TR, Herzog W. Residual force enhancement in myofibrils and sarcomeres. *Proc Biol Sci* 2008;**275**:1411–9.
35. Herzog W. Mechanisms of enhanced force production in lengthening (eccentric) muscle contractions. *J Appl Physiol* 2014;**116**:1407–17.
36. Herzog W. The role of titin in eccentric muscle contraction. *J Exp Biol* 2014;**217**:2825–83.
37. Pun C, Syed A, Rassier DE. History-dependent properties of skeletal muscle myofibrils contracting along the ascending limb of the force-length relationship. *Proc Biol Sci* 2010;**277**:475–84.
38. Leonard TR, DuVall M, Herzog W. Force enhancement following stretch in a single sarcomere. *Am J Physiol Cell Physiol* 2010;**299**:C1398–401.
39. Telley IA, Stehle R, Ranatunga KW, Pfitzer G, Stussi E, Denoth J. Dynamic behaviour of half-sarcomeres during and after stretch in activated rabbit psoas myofibrils: sarcomere asymmetry but no "sarcomere popping". *J Physiol* 2006;**573**:173–85.
40. Rassier DE. The mechanisms of the residual force enhancement after stretch of skeletal muscle: non-uniformity in half-sarcomeres and stiffness of titin. *Proc Biol Sci* 2012;**279**:2705–13.
41. Stoecker U, Telley IA, Stussi E, Denoth J. A multisegmental cross-bridge kinetics model of the myofibril. *J Theor Biol* 2009;**259**:714–26.
42. Campbell SG, Hatfield PC, Campbell KS. A mathematical model of muscle containing heterogeneous half-sarcomeres exhibits residual force enhancement. *PLoS Comput Biol* 2011;**7**: e1002156. doi:10.1371/journal.pcbi.1002156.
43. Campbell SG, Campbell KS. Mechanisms of residual force enhancement in skeletal muscle: insights from experiments and mathematical models. *Biophys Rev* 2011;**3**:199–207.
44. Huxley H, Hanson J. Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature* 1954;**173**:973–6.
45. Hanson J, Huxley HE. Quantitative studies on the structure of cross-striated myofibrils. II. Investigations by biochemical techniques. *Biochim Biophys Acta* 1957;**23**:250–60.
46. Rall JA. *Mechanism of muscular contraction*. New York, NY: Springer; 2014.
47. Dos Remedios C, Gilmour D. An historical perspective of the discovery of titin filaments. *Biophys Rev* 2017;**9**:179–88.
48. McNeill PF, Hoyle G. Evidence for superthin filaments. *Am Zoologist* 1967;**7**:483–98.
49. Huxley HE. Structural difference between resting and rigor muscle; evidence from intensity changes in the low angle equatorial X-ray diagram. *J Mol Biol* 1968;**37**:507–20.
50. Lindstedt SL, Nishikawa K. Huxleys' missing filament: form and function of titin in vertebrate skeletal muscle. *Ann Rev Physiol* 2017;**79**:145–66.
51. Sjostrand FS. The connections between A- and I-band filaments in striated frog muscle. *Annu Rev Physiol* 1962;**7**:225–46.
52. Maruyama K. Connectin, an elastic protein from myofibrils. *J Biochem* 1976;**80**:405–7.
53. Wang K, McClure J, Tu A. Titin: major myofibrillar components of striated muscle. *Proc Natl Acad Sci U S A* 1979;**76**:3698–702.
54. Maruyama K, Kimura S, Ohashi K, Kuwano Y. Connectin, an elastic protein of muscle. Identification of "titin" with connectin. *J Biochem* 1981;**89**:701–9.
55. Fürst DO, Osborn M, Nave R, Weber K. The organization of titin filaments in the half-sarcomere revealed by monoclonal antibodies in

immunoelectron microscopy: a map of ten nonrepetitive epitopes starting at the Z line extends close to the M line. *J Cell Biol* 1988;**106**:1563–72.
56. Yamada A, Yoshio M, Kojima H, Oiwa K. An *in vitro* assay reveals essential protein components for the "catch" state of invertebrate smooth muscle. *Proc Natl Acad Sci U S A* 2001;**98**:6635–40.
57. Gregorio CC, Granzier H, Sorimachi H, Labeit S. Muscle assembly: a titanic achievement. *Curr Opin Cell Biol* 1999;**11**:18–25.
58. Labeit S, Kolmerer B. Titins: giant proteins in charge of muscle ultrastructure and elasticity. *Science* 1995;**270**:293–6.
59. Gautel M, Goulding D. A molecular map of titin/connectin elasticity reveals two different mechanisms acting in series. *FEBS Lett* 1996;**385**:11–4.
60. Linke WA, Bartoo ML, Ivemeyer M, Pollack GH. Limits of titin extension in single cardiac myofibrils. *J Muscle Res Cell Motil* 1996;**17**:425–38.
61. Linke WA, Ivemeyer M, Mundel P, Stockmeier MR, Kolmerer B. Nature of PEVK-titin elasticity in skeletal muscle. *Proc Natl Acad Sci U S A* 1998;**95**:8052–7.
62. Kellermayer MS, Granzier HL. Elastic properties of single titin molecules made visible through fluorescent F-actin binding. *Biochem Biophys Res Commun* 1996;**221**:491–7.
63. Maruyama K, Natori R, Nonomura Y. New elastic protein from muscle. *Nature* 1976;**262**:58–60.
64. Horowits R, Kempner ES, Bisher ME, Podolsky RJ. A physiological role for titin and nebulin in skeletal muscle. *Nature* 1986;**323**:160–4.
65. Horowits R, Podolsky RJ. The positional stability of thick filaments in activated skeletal muscle depends on sarcomere length: evidence for the role of titin filaments. *J Cell Biol* 1987;**105**:2217–23.
66. Linke WA. Titin gene and protein functions in passive and active muscle. *Annu Rev Physiol* 2018;**80**:389–411.
67. Konhilas JP, Irving TC, de Tombe PP. Length-dependent activation in three striated muscle types of the rat. *J Physiol* 2002;**544**:225–36.
68. Mateja RD, Greaser ML, de Tombe PP. Impact of titin isoform on length dependent activation and cross-bridge cycling kinetics in rat skeletal muscle. *Biochim Biophys Acta* 2013;**1833**:804–11.
69. Irving T, Wu Y, Bekyarova T, Farman GP, Fukuda N, Granzier H. Thick-filament strain and interfilament spacing in passive muscle: effect of titin-based passive tension. *Biophys J* 2011;**100**:1499–508.
70. Edman KA, Elzinga G, Noble MI. Proceedings: force enhancement induced by stretch of contracting single isolated muscle fibres of the frog. *J Physiol* 1976;**258**(2):95P–96P.
71. Edman KA, Tsuchiya T. Strain of passive elements during force enhancement by stretch in frog muscle fibres. *J Physiol* 1996;**490**:191–205.
72. Herzog W, Leonard TR. Force enhancement following stretching of skeletal muscle: a new mechanism. *J Exp Biol* 2002;**205**:1275–83.
73. Joumaa V, Rassier DE, Leonard TR, Herzog W. The origin of passive force enhancement in skeletal muscle. *Am J Physiol Cell Physiol* 2008;**294**:C74–8.
74. Bagni MA, Cecchi G, Colombini B, Colomo F. A non-cross-bridge stiffness in activated frog muscle fibers. *Biophys J* 2002;**82**:3118–27.
75. Bagni MA, Colombini B, Geiger P, Berlinguer Palmini R, Cecchi G. Non-cross-bridge calcium-dependent stiffness in frog muscle fibers. *Am J Physiol Cell Physiol* 2004;**286**:C1353–7.
76. Nocella M, Cecchi G, Bagni MA, Colombini B. Force enhancement after stretch in mammalian muscle fiber: no evidence of cross-bridge involvement. *Am J Physiol Cell Physiol* 2014;**307**:C1123–9.
77. Rassier DE, Leite FS, Nocella M, Cornachione AS, Colombini B, Bagni MA. Non-crossbridge forces in activated striated muscles: a titin dependent mechanism of regulation? *J Muscle Res Cell Motil* 2015;**36**:37–45.
78. Leonard TR, Herzog W. Regulation of muscle force in the absence of actin-myosin-based cross-bridge interaction. *Am J Physiol Cell Physiol* 2010;**299**:C14–20.
79. Powers K, Schappacher-Tilp G, Jinha A, Leonard T, Nishikawa K, Herzog W. Titin force is enhanced in actively stretched skeletal muscle. *J Exp Biol* 2014;**217**:3629–36.
80. Granzier HL. Activation and stretch-induced passive force enhancement-are you pulling my chain? Focus on "Regulation of muscle force in the absence of actin-myosin-based cross-bridge interaction". *Am J Physiol Cell Physiol* 2010;**299**:C11–3.
81. Herzog W, Schappacher G, DuVall M, Leonard TR, Herzog JA. Residual force enhancement following eccentric contractions: a new mechanism involving titin. *Physiology* 2016;**31**:300–12.
82. Labeit D, Watanabe K, Witt C, Fujita H, Wu Y, Lahmers S, et al. Calcium-dependent molecular spring elements in the giant protein titin. *Proc Natl Acad Sci U S A* 2003;**100**:13716–21.
83. Kellermayer MS, Granzier HL. Calcium-dependent inhibition of *in vitro* thin-filament motility by native titin. *FEBS Lett* 1996;**380**:281–6.
84. Rode C, Siebert T, Blickhan R. Titin-induced force enhancement and force depression: a "sticky-spring" mechanism in muscle contractions. *J Theor Biol* 2009;**259**:350–60.
85. Nishikawa KC, Monroy JA, Uyeno TE, Yeo SH, Pai DK, Lindstedt SL. Is titin a "winding filament"? A new twist on muscle contraction. *Proc Biol Sci* 2012;**279**:981–90.
86. Powers K, Nishikawa K, Joumaa V, Herzog W. Decreased force enhancement in skeletal muscle sarcomeres with a deletion in titin. *J Exp Biol* 2016;**219**:1311–6.
87. Schappacher-Tilp G, Leonard T, Desch G, Herzog W. A novel three-filament model of force generation in eccentric contraction of skeletal muscles. *PLoS One* 2015;**10**: e0117634. doi:10.1371/journal.pone.0117634.
88. Hessel AL, Nishikawa KC. Effects of a titin mutation on negative work during stretch-shortening cycles in skeletal muscles. *J Exp Biol* 2017;**220**:4177–85.
89. Linke WA, Ivemeyer M, Labeit S, Hinssen H, Ruegg JC, Gautel M. Actin-titin interaction in cardiac myofibrils: probing a physiological role. *Biophys J* 1997;**73**:905–19.
90. Linke WA, Kulke M, Li H, Fujita-Becker S, Neagoe C, Manstein DJ, et al. PEVK domain of titin: an entropic spring with actin-binding properties. *J Struct Biol* 2002;**137**:194–205.
91. Linke WA, Fernandez JM. Cardiac titin: molecular basis of elasticity and cellular contribution to elastic and viscous stiffness components in myocardium. *J Muscle Res Cell Motil* 2002;**23**:483–97.
92. Yamasaki R, Berri M, Wu Y, Trombitas K, McNabb M, Kellermayer MS, et al. Titin-actin interaction in mouse myocardium: passive tension modulation and its regulation by calcium/S100A1. *Biophys J* 2001;**81**:2297–313.
93. Garvey SM, Rajan C, Lerner AP, Frankel WN, Cox GA. The muscular dystrophy with myositis (mdm) mouse mutation disrupts a skeletal muscle-specific domain of titin. *Genomics* 2002;**79**:146–9.
94. DuVall MM, Jinha A, Schappacher-Tilp G, Leonard TR, Herzog W. Differences in titin segmental elongation between passive and active stretch in skeletal muscle. *J Exp Biol* 2017;**220**:4418–25.
95. Hooper SL, Hobbs KH, Thuma JB. Invertebrate muscles: thin and thick filament structure; molecular basis of contraction and its regulation, catch and asynchronous muscle. *Prog Neurobiol* 2008;**86**:72–127.
96. Sugi H, Suzuki S. The nature of potassium- and acetylcholine-induced contractures in the anterior byssal retractor muscle of *Mytilus edulis*. *Comp Biochem Physiol C* 1978;**61**:275–9.
97. Kenny PA, Liston EM, Higgins DG. Molecular evolution of immunoglobulin and fibronectin domains in titin and related muscle proteins. *Gene* 1999;**232**:11–23.
98. Butler TM, Siegman MJ. Mechanism of catch force: tethering of thick and thin filaments by twitchin. *J Biomed Biotechnol* 2010;**2010**: 725207. doi:10.1155/2010/725207.
99. Tameyasu T, Sugi H. The series elastic component and the force-velocity relation in the anterior byssal retractor muscle of Mytilus edulis during active and catch contractions. *J Exp Biol* 1976;**64**:497–510.
100. Takahashi M, Sohma H, Morita F. The steady state intermediate of scallop smooth muscle myosin ATPase and effect of light chain phosphorylation. A molecular mechanism for catch contraction. *J Biochem* 1988;**104**:102–7.
101. Butler TM, Siegman MJ. Control of cross-bridge cycling by myosin light chain phosphorylation in mammalian smooth muscle. *Acta Physiol Scand* 1998;**164**:389–400.
102. Butler TM, Mooers SU, Li C, Narayan S, Siegman MJ. Regulation of catch muscle by twitchin phosphorylation: effects on force, ATPase, and shortening. *Biophys J* 1998;**75**:1904–14.

103. Funabara D, Watabe S. The catch mechanism of anterior bosses retractor muscle in mussel. *Nippon Suisan Gakk* 2002;**68**:913–4.
104. Funabara D, Kanoh S, Siegman MJ, Butler TM, Hartshorne DJ, Watabe S. Twitchin as a regulator of catch contraction in molluscan smooth muscle. *J Muscle Res Cell Motil* 2005;**26**:455–60.
105. Shelud'ko NS, Matusovskaya GG, Permyakova TV, Matusovsky OS. Twitchin, a thick-filament protein from molluscan catch muscle, interacts with F-actin in a phosphorylation-dependent way. *Arch Biochem Biophys* 2004;**432**:269–77.
106. Hessel AL, Lindstedt SL, Nishikawa KC. Physiological mechanisms of eccentric contraction and its applications: a role for the giant titin protein. *Front Physiol* 2017;**8**:70. doi:10.3389/fphys.2017.00070.
107. Edwards RH, Newham DJ, Jones DA, Chapman SJ. Role of mechanical damage in pathogenesis of proximal myopathy in man. *The Lancet* 1984;**1**:548–52.
108. Newham DJ, Jones DA, Ghosh G, Aurora P. Muscle fatigue and pain after eccentric contractions at long and short length. *Clin Sci (Lond)* 1988;**74**:553–7.
109. Flann KL, LaStayo PC, McClain DA, Hazel M, Lindstedt SL. Muscle damage and muscle remodeling: no pain, no gain. *J Exp Biol* 2011;**214**:674–9.
110. Hoppeler H, Herzog W. Eccentric exercise: many questions unanswered. *J Appl Physiol* 2014;**116**:1405–6.
111. LaStayo P, McDonagh P, Lipovic D, Napoles P, Bartholomew A, Esser K, et al. Elderly patients and high force resistance exercise—a descriptive report: can an anabolic, muscle growth response occur without muscle damage or inflammation. *J Geriatr Phys Ther* 2007;**30**:128–34.
112. LaStayo PC, Ewy GA, Pierotti DD, Johns RK, Lindstedt S. The positive effects of negative work: increased muscle strength and decreased fall risk in a frail elderly population. *J Gerontol A Biol Sci Med Sci* 2003;**58**:M419–24.
113. Kraemer WJ, Adams K, Cafarelli E, Dudley GA, Dooly C, Feigenbaum MS, et al. American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. *Med Sci Sports Exerc* 2002;**34**:364–80.
114. LaStayo PC, Pierotti DJ, Pifer J, Hoppeler H, Lindstedt SL. Eccentric ergometry: increases in locomotor muscle size and strength at low training intensities. *Am J Physiol Regul Integr Comp Physiol* 2000;**278**:R1282–8.
115. LaStayo PC, Reich TE, Urquhart M, Hoppeler H, Lindstedt SL. Chronic eccentric exercise: improvements in muscle strength can occur with little demand for oxygen. *Am J Physiol* 1999;**276**:R611–5.
116. Meyer A, Tumilty S, Baxter GD. Eccentric exercise protocols for chronic non-insertional Achilles tendinopathy: how much is enough. *Scand J Med Sci Sports* 2009;**19**:609–15.
117. Hoppeler H. Moderate load eccentric exercise; a distinct novel training modality. *Front Physiol* 2016;**7**:483. doi:10.3389/fphys.2016.00483.
118. LaStayo P, Marcus R, Dibble L, Frajacomo F, Lindstedt S. Eccentric exercise in rehabilitation: safety, feasibility, and application. *J Appl Physiol* 2014;**116**:1426–34.
119. Meyer K, Steiner R, Lastayo P, Lippuner K, Allemann Y, Eberli F, et al. Eccentric exercise in coronary patients: central hemodynamic and metabolic responses. *Med Sci Sports Exerc* 2003;**35**:1076–82.
120. Besson D, Joussain C, Gremeaux V, Morisset C, Laurent Y, Casillas JM, et al. Eccentric training in chronic heart failure: feasibility and functional effects. Results of a comparative study. *Ann Phys Rehabil Med* 2013;**56**:30–40.
121. Rocha Vieira DS, Baril J, Richard R, Perrault H, Bourbeau J, Taivassalo T. Eccentric cycle exercise in severe COPD: feasibility of application. *COPD* 2011;**8**:270–4.
122. Fluck M, Bosshard R, Lungarella M. Cardiovascular and muscular consequences of work-matched interval-type of concentric and eccentric pedaling exercise on a soft robot. *Front Physiol* 2017;**8**:640. doi:10.3389/fphys.2017.00640.
123. Steiner R, Meyer K, Lippuner K, Schmid JP, Saner H, Hoppeler H. Eccentric endurance training in subjects with coronary artery disease: a novel exercise paradigm in cardiac rehabilitation. *Eur J Appl Physiol* 2004;**91**:572–8.
124. Theodorou AA, Panayiotou G, Paschalis V, Nikolaidis MG, Kyparos A, Mademli L, et al. Stair descending exercise increases muscle strength in elderly males with chronic heart failure. *BMC Res Notes* 2013;**6**:87. doi:10.1186/1756-0500-6-87.
125. Gremeaux V, Duclay J, Deley G, Philipp JL, Laroche D, Pousson M, et al. Does eccentric endurance training improve walking capacity in patients with coronary artery disease? A randomized controlled pilot study. *Clin Rehabil* 2010;**24**:590–9.
126. Casillas JM, Besson D, Hannequin A, Gremeaux V, Morisset C, Tordi N, et al. Effects of an eccentric training personalized by a low rate of perceived exertion on the maximal capacities in chronic heart failure: a randomized controlled trial. *Eur J Phys Rehabil Med* 2016;**52**:159–68.
127. LaStayo PC, Marcus RL, Dibble LE, Smith SB, Beck SL. Eccentric exercise versus usual-care with older cancer survivors: the impact on muscle and mobility-an exploratory pilot study. *BMC Geriatr* 2011;**11**:5. doi:10.1186/1471-2318-11-5.
128. Dibble LE, Addison O, Papa E. The effects of exercise on balance in persons with Parkinson's disease: a systematic review across the disability spectrum. *J Neurol Phys Ther* 2009;**33**:14–26.
129. Gerber JP, Marcus RL, Dibble LE, Greis PE, Burks RT, LaStayo PC. Effects of early progressive eccentric exercise on muscle structure after anterior cruciate ligament reconstruction. *J Bone Joint Surg Am* 2007;**89**:559–70.
130. Gerber JP, Marcus RL, Leland ED, Lastayo PC. The use of eccentrically biased resistance exercise to mitigate muscle impairments following anterior cruciate ligament reconstruction: a short review. *Sports Health* 2009;**1**:31–8.
131. LaStayo PC, Meier W, Marcus RL, Mizner R, Dibble L, Peters C. Reversing muscle and mobility deficits 1 to 4 years after TKA: a pilot study. *Clin Orthop Relat Res* 2009;**467**:1493–500.
132. Marcus RL, Lastayo PC, Dibble LE, Hill L, McClain DA. Increased strength and physical performance with eccentric training in women with impaired glucose tolerance: a pilot study. *J Womens Health (Larchmt)* 2009;**18**:253–60.
133. Marcus RL, Smith S, Morrell G, Addison O, Dibble LE, Wahoff-Stice D, et al. Comparison of combined aerobic and high-force eccentric resistance exercise with aerobic exercise only for people with type 2 diabetes mellitus. *Phys Ther* 2008;**88**:1345–54.
134. van Melick N, van Cingel RE, Brooijmans F, Neeter C, van Tienen T, Hullegie W, et al. Evidence-based clinical practice update: practice guidelines for anterior cruciate ligament rehabilitation based on a systematic review and multidisciplinary consensus. *Br J Sports Med* 2016;**50**:1506–15.
135. Hortobagyi T. The positives of negatives: clinical implications of eccentric resistance exercise in old adults. *J Gerontol A Biol Sci Med Sci* 2003;**58**:M417–8.
136. LaStayo P, Marcus R, Dibble L, Wong B, Pepper G. Eccentric versus traditional resistance exercise for older adult fallers in the community: a randomized trial within a multi-component fall reduction program. *BMC Geriatrics* 2017;**17**:149. doi:10.1186/s12877-017-0539-8.
137. Eldridge N, Gould SJ. Punctuated equilibria: an alternative to phyletic gradualism. In: Schopf TJM, editor. *Models in paleobiology*. San Francisco, CA: Freeman Cooper; 1972.p.82–115.