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REVIEW

Skeletal muscle tissue in movement and health: positives and negatives

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ABSTRACT

The history of muscle physiology is a wonderful lesson in 'the scientific method'; our functional hypotheses have been limited by our ability to decipher (observe) muscle structure. The simplistic understanding of how muscles work made a large leap with the remarkable insights of A. V. Hill, who related muscle force and power to shortening velocity and energy use. However, Hill's perspective was largely limited to isometric and isotonic contractions founded on isolated muscle properties that do not always reflect how muscles function *in vivo*. Robert Josephson incorporated lengthening contractions into a work loop analysis that shifted the focus to dynamic muscle function, varying force, length and work done both by and on muscle during a single muscle work cycle. It became apparent that muscle is both a force generator and a spring. Titin, the missing filament in the sliding filament model, is a muscle spring, which functions very differently in cardiac versus skeletal muscle; its possible role in these two muscle types is discussed relative to their contrasting function. The good news for those of us who choose to work on skeletal muscle is that muscle has been reluctant to reveal all of its secrets.

KEY WORDS: Hill plot, Work loop, Fenn effect, Eccentric, Titin

Introduction

Skeletal muscle makes up 40% of an organism's body mass and is the sink for nearly all the oxygen an animal consumes during maximum sustained exertion. Historically considered to be composed of genetically predetermined fiber types, the phenotypic plasticity of skeletal muscle, responding to both the nature and magnitude of the force-producing demands, is now appreciated. Rather than genetically inflexible, one might conclude that, 'muscle is what muscle does'. Remarkably, shifts in the relative proportions of just three basic components (myofibrils, mitochondria and sarcoplasmic reticulum) are responsible for a diversity of functions including noisemaking, posture, endurance, ballistic movements and thermogenesis (Schaeffer and Lindstedt, 2013).

The history of muscle physiology is an excellent example of what we teach as the 'scientific method'; the merit of any hypothesis reflects the limitations of observation. Thus, the earliest observation that nerves are somehow connected to muscles and muscles increase in cross-sectional area when they contract resulted in a functional hypothesis that survived for centuries. Pneuma was thought to be transmitted through the nerves to 'fill' the muscles, causing shortening. This idea survived until the 17th century when the British physician Glisson used Archimedes' principle to demonstrate that contracting muscle did not change in volume. This observation prompted the Swiss scientist von Haller (credited as the 'Father of Neurobiology') to suggest that it was irritability, not a humor, which is transmitted to the muscle through the nerve. For a wonderful comprehensive examination of muscle history, the definitive source is the book '*Machina Carnis*' by Needham (1971).

The first Professor of Physiology in the USA (Columbia University) was the Civil War surgeon J. C. Dalton, who authored the first USA textbook of physiology ('*Treatise on Human Physiology*'). He observed that irritability (which he noted could be triggered with an electric shock) is an inherent property of the muscle fiber, 'not communicated to it by other parts' (Dalton, 1864). The consequence of this 'irritability' is that muscles produce force when they contract.

The understanding of muscle physiology had not progressed much beyond Dalton's insights when A. V. Hill was born in 1886. Hill's groundbreaking experiments, beginning in 1910, yielded a Nobel Prize (shared with Otto Meyerhof) in 1922 when he was just 36 years of age. Those early studies and insights also resulted in several enduring principles that still occupy the first pages of any muscle physiology text. Bassett (2002) provides an entertaining and educational review of Hill's life and contributions.

Muscle force, velocity, power and energy cost

Among Hill's many contributions, none have been more enduring than the force–velocity curve (Hill, 1938). The hyperbolic relationship of force and velocity that he described has become the foundation on which subsequent muscle discoveries have been built. It is now accepted that muscles produce maximum force only if there is no shortening, and produce maximum velocity when shortening occurs at (theoretical) zero force. While his experiments preceded the concept of 'fiber types' by decades, these early experiments identified two core principles. First, although muscle fibers differ greatly in their maximum velocity (V_{max}), all fibers produce roughly the same maximum isometric force per unit cross-sectional area ($\mathbf{F}_{max}$). Second, because the product of force and velocity is power, these two measurements yield yet another variable and another fundamental property of muscle. Because of the predictable hyperbolic shape of the force–velocity curve, all muscles produce their maximum power (P_{max}) at a shortening velocity of about one-third V_{max}. These relationships, as well as many entertaining *in vivo* consequences, are described in an engaging classic paper based on a Friday evening lecture Hill gave to the Royal Society, highly recommended reading for any serious student of muscle physiology (Fig. 1) (Hill, 1950).

Perhaps in part because of limits in early 20th century technology, the principles and properties of isometric and isotonic contractions of muscle seemed to adequately describe the suite of muscle force production *in vivo*. However, in addition to isometric contractions (no change in length) and shortening contractions (performing work), muscle often is stretched while contracting (work being done on the muscle). While Hill acknowledged that

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active muscles are often stretched, there was such a dearth of understanding of lengthening contractions that 50 years after Hill's discoveries, the late Bioengineer Tom McMahon referred to lengthening muscle contractions as the 'dark side of the force–velocity curve' (Lindstedt et al., 2001). Indeed, it was Wallace Fenn, Hill's 'postdoc' (not a term used at the time) who examined all three modes of contraction in his work coupling muscle energetics with mechanics. What is now known as the 'Fenn effect' is that muscle requires more energy if doing work (shortening) than is required for the same magnitude of force production if no work is done (isometric contraction). However, Fenn also made a second, less commonly discussed, observation: if work is done on the muscle (a lengthening contraction), less energy is required compared with an isometric contraction, functionally a 'negative Fenn effect' (Fenn, 1924).

We recently tested the Fenn effect quantitatively (Ortega et al., 2015). By coupling magnetic resonance spectroscopy with *in vivo* length and force measurements in the first dorsal interosseous (FDI) muscle in humans, we were able to control the magnitude and duration of force production. Under these conditions, muscle movement (shortening, constant length or lengthening) is the only experimental variable as both the magnitude and duration of force production (the force–time integral, FTI) are held constant. In all cases, the energy cost was a linear function of the load (Fig. 2A). These experiments controlled velocity and revealed that the energy cost was highest when the muscle shortened and quantitatively equal to the isometric cost plus work done by the FDI muscle. The cost of force production was the least when the muscle was stretched (Fig. 2B) (Ortega et al., 2015). The minimal energy difference observed in lengthening contractions may have been increased if the muscle had been actively, rather than passively stretched. The simple interpretation is that if muscle is held to a constant length or if it is stretched, the energy cost of producing force is greatly reduced relative to a shortening contraction, suggesting that an elastic element 'partners' with cross-bridges to reduce the cost of force production.

The 'high energy–low cost' of lengthening contractions was first dramatically demonstrated by Hill's PhD student Bernard 'Bud' Abbott using mechanically linked back-to-back stationary bicycles. Under these conditions, much less effort and energy was required to resist than to propel the pedals (Abbott et al., 1952). A review of this classic experiment was a recent feature in this journal (Elmer and LaStayo, 2014). This observation triggered an interesting series of futile investigations by none other than Hill to demonstrate that the processes that consume ATP in shortening muscle can be reversed when the muscle is stretched, generating ATP (Hill and Howarth, 1959; Hill, 1960). I include this anecdote as a reminder that the key to success is risking failure, which even the great ones must experience. Thus, while stretching muscle is no longer considered to function as an 'ATP generator', the processes responsible for the reduced energy cost of force production in actively stretched muscle remain unclear.

Lengthening muscle contractions in normal locomotion

Any time the force applied to a muscle exceeds the force generated by the muscle, the muscle will be stretched while activated. Initially, this kind of contraction was labeled 'excentric' or away from the center, by Asmussen (1953), though this later evolved into the term 'eccentric', which makes little sense (Faulkner, 2003) yet has gained acceptance in the literature. Because both force and distance are vectors, they must be defined by both magnitude and direction. For that reason, when the force produced by the muscle is opposite

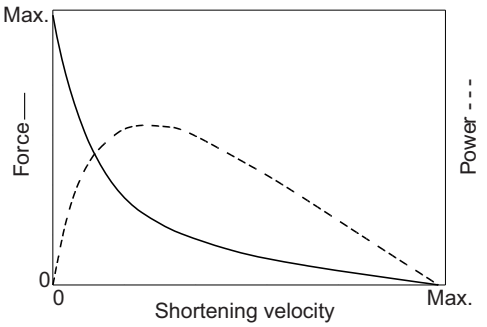

Fig. 1. Hill's pioneering experiments provided the force–velocity curve shown here. Because power is the product of force and velocity, these two measurements yield one more variable. Adapted from Hill (1950).

in direction to the change in muscle length, the simple work equation becomes: $\mathbf{F}\times-\mathbf{D}=-W$, with the result that eccentric contractions produce, by definition, 'negative work'. There are two possible outcomes for negative work (i.e. work done on the muscle): (1) the absorbed energy can be lost as heat, as when walking downhill, or (2) the absorbed energy can be stored as elastic recoil potential energy, some of which can be recovered. In fact, in almost all instances locomotor muscles are used cyclically, and are subject to sequential stretch and shortening, which allows animals to recover stored elastic recoil energy.

Changing our view from isolated (static) to *in vivo* (dynamic) properties of muscle, Robert Josephson (Josephson and Young, 1981) introduced the concept of the work loop (Fig. 3). Importantly, not only do muscles produce force but they also (with their tendon partners) store absorbed energy and recover it with each stride (see, for example, Biewener and Roberts, 2000). For example, in running birds and mammals, during each stride, from footfall until the center of mass is over the foot, extensors of the hip, knee and ankle are stretched while 'contracting'. The subsequent recovery of the stored elastic energy greatly reduces the cost of movement. Linari et al. (2003) found that up to 56% of the work done to stretch an isolated active fiber can be stored and recovered. Because this storage and recovery is strongly time dependent (Lindstedt et al., 2001), galloping mammals apparently select stride frequencies to maximize the recovery of elastic recoil energy (Schaeffer and Lindstedt, 2013). This link is one lesson from a student lab. Self-selected hopping frequency is identical to the body-size predicted stride frequency for galloping mammals, and if forced to shift that frequency, the cost per hop doubles (Lindstedt et al., 2013).

Lengthening muscle contractions as an intervention

Despite the key, undisputed, finding that actively lengthening muscle produces the most force and does so at a greatly reduced energy cost, surprisingly little attention was given to employing lengthening contractions as an intervention. Individuals suffering from sarcopenia or other pathologies (e.g. chronic heart failure, chronic obstructive pulmonary disease) in which muscle atrophy is either a causal or exacerbating trigger, could specifically benefit from the high force and low energy cost of eccentric contractions (Hortobagyi, 2003). However, progress in this area was greatly retarded by the belief that there was a third property of lengthening contractions; eccentric contractions cause muscle damage, especially in older adults (for review, see Clarkson and Hubal, 2002). Sadly, this unfortunate association became viewed as an obligate link that made this kind of exercise not only undesirable but also one that should be avoided because of its perceived high risk,

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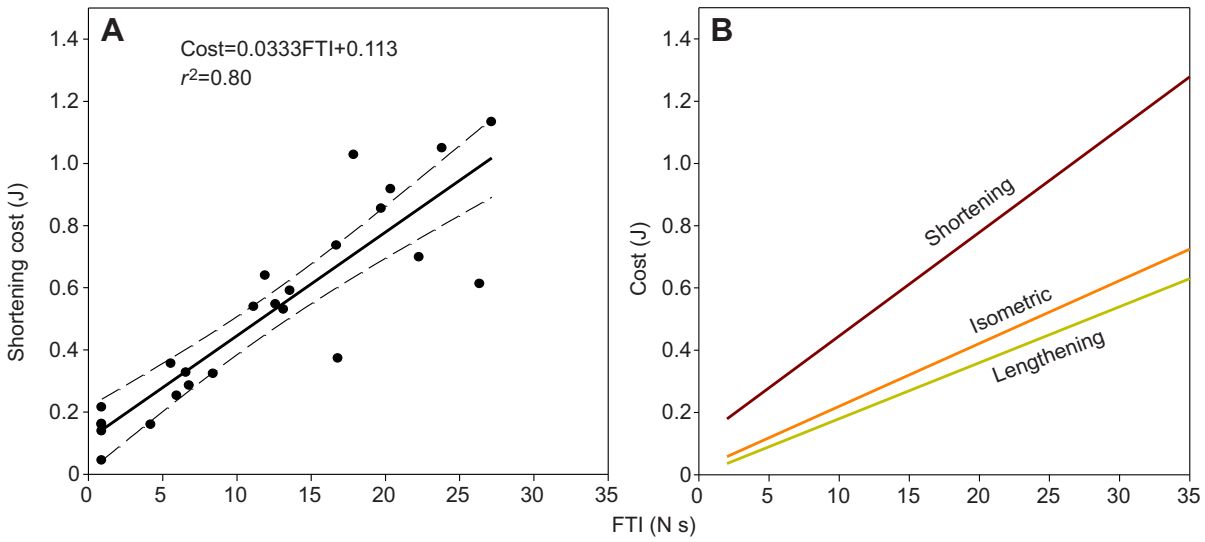

Fig. 2. Energy cost of force production in the first dorsal interosseous (FDI) muscle. (A) The energy cost of a muscle contraction increases linearly as a function of the magnitude and duration of the force produced, the force–time integral (FTI). (B) At all loads (FTIs), the highest energy cost occurs when the muscle is required to do work while shortening. Less energy is required if the muscle does not shorten and the least energy requirement occurs if the muscle contracts eccentrically. Reprinted from Ortega et al. (2015).

especially for frail adults. Often in science, we are eager to accept conclusions with minimal critical analysis – far less evidence is required to establish an idea as 'fact' than is required to dislodge that idea once established (the 'sufficiency of proof axiom', *sensu* Lindstedt et al., 2001). Such was the link between eccentric contractions and muscle damage.

In fact, muscles frequently experience lengthening contractions, which are as common in our daily activities as shortening contractions. It is not lengthening contractions per se that are damaging, but rather novel, high-force lengthening contractions that can cause damage. By ramping up the workload slowly, it is possible for even the frailest individuals to benefit from chronic 'eccentric' training. We engaged a group of individuals, all at high risk for falling, to examine whether chronic eccentric training, i.e. negative work, could function as an intervention to limit or even reverse sarcopenia and its related impairments and functional limitations. Twenty-one frail elderly subjects (mean age, 80 years) participated in 11 weeks of lower extremity resistance training. The experimental group performed negative work while exercising on a high-force eccentric ergometer. The active 'controls' performed traditional lower extremity weight training. Muscle fiber cross-sectional area and strength, balance, stair-descending ability and fall risk were assessed prior to and following this intervention. All subjects who started the negative work intervention completed the study and reported the training to be relatively effortless; they experienced minimal and transient muscle soreness. Both groups experienced a significant increase in muscle fiber cross-sectional area but only the experimental group experienced significant improvements in strength (60%), balance (7%) and stair descent (21%) abilities. The 'timed up and

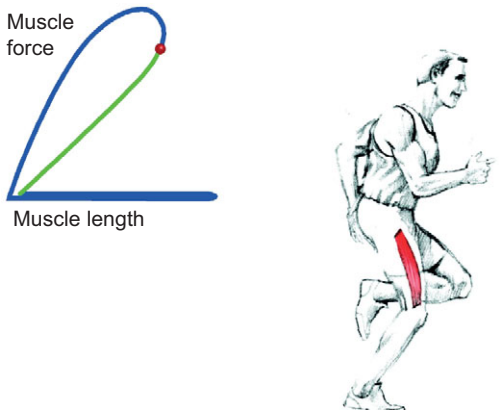

Fig. 3. Work loop traces the force produced by the muscle and its length during one contraction cycle. Because the product of force and length is work, the area in the work loop represents the work done by the muscle. Locomotor muscles used cyclically have an active lengthening phase, from footfall until the center of mass is directly above the foot (shown here). The work done on the muscle (stretching it) can be stored and contribute to the force produced during muscle shortening, saving energy. Reprinted from Schaeffer and Lindstedt (2013).

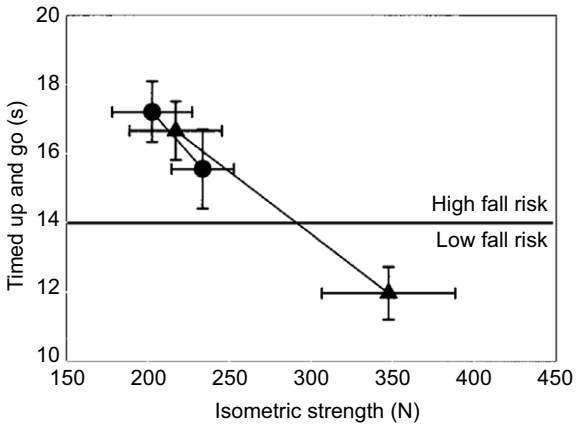

Fig. 4. The 'timed up and go' test is a predictor of fall risk in humans. Here, the time before and after an eccentric intervention (triangles) is shown along with the weight-lifting controls (circles). All subjects were at high fall risk to qualify for the study. Those who participated in the eccentric training had a much improved time following the intervention, as well as improvements in a number of tests of function and balance. Reprinted from LaStayo et al. (2003) with permission from Oxford University Press [**LaStayo, P. C., Ewy, G., Pierotti, D., Johns, R. and Lindstedt, S. L.** (2003). 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.* **58**, M419-M424].

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go' task improved in both groups, but only the experimental group went from a high to a low fall risk (Fig. 4). For this group of frail elders, a chronic negative work intervention resulted in increased muscle structure and function in individuals limited to relatively 'low intensity' exercise (LaStayo et al., 2003).

Titin: Huxleys' missing filament

Compared with isometric contractions, muscles: (1) produce greater force with lower metabolic cost when stretched and (2) produce less force and require more energy when shortening. Thus, muscle fibers behave as though there is an elastic element in series with the thick and thin filaments. When the sliding filament theory was proposed (Huxley and Niedergerke, 1954; Huxley and Hanson, 1954) it incorporated all known structural proteins within the sarcomere and accounted for the Hill muscle mechanics. However, the behavior of muscle stretch has required modifications to the simple sliding filaments (reviewed in this issue by Nishikawa, 2016). In fact, unknown to the Huxleys, there was a third major filament residing within the sarcomere, the largest known protein, titin. Discovered in 1976 (Maruyama et al., 1976), titin is the largest known protein and it is found in virtually all vertebrate and invertebrate striated muscle (Bullard et al., 2002). Its ubiquitous presence argues strongly for a pivotal functional role, which early on was thought to be passive muscle tension (Linke et al., 1998). Both its structure and location, spanning the half sarcomere from the Z-disk to the thick filament, suggest to many that it functions as an internal sarcomere 'spring' capable of storing elastic potential energy (e.g. Reich et al., 2000; Herzog and Leonard, 2002; Nishikawa et al., 2012).

Cardiac titin – a stiff spring

The roles of titin as an internal elastic element (i.e. muscle spring) have been best described in cardiac muscle. In particular, the mechanism of increased ventricular contractility with increased preload, the 'Frank–Starling law of the heart', is an observation that has lacked a mechanistic explanation. Titin's elastic properties have been implicated as a key to this phenomenon (Fukuda and Granzier, 2004). Among the purported roles of titin in cardiac muscle are the following: (1) titin has a key function in stress-sensing signaling (Anderson and Granzier, 2012); (2) titin passive tension triggers an increase in Ca^{2+} sensitivity and thus increased contractility (Lee et al., 2010); (3) titin is involved in length-dependent thin filament regulation (Kobirumaki-Shimozawa et al., 2014); and finally (4) titin functions "as a 'bidirectional spring' that … determines not only ventricular rigidity and diastolic function, but also systolic cardiac function" (Castro-Ferreira et al., 2011). In other words, once this 'undiscovered' filament found its place in cardiac muscle, a number of elusive features of cardiac muscle gained clarity. Importantly, all of these stated roles of titin can be attributed to the fact that the passive stiffness of titin is tuned to the *in vivo* length changes in ventricular sarcomeres that occur during normal diastole (Fig. 5) (Neagoe et al., 2003).

Skeletal muscle titin – a compliant bungee

The titin isoform in skeletal muscle occupies the same structural relationship within the sarcomere as it does in cardiac muscle and it too exerts a passive stiffness when stretched. However, skeletal muscle titin is composed of many more tandem Ig (immunoglobulin) and PEVK (named for its most prevalent amino acids) domains, functionally resulting in a longer and thus much more compliant molecule. When skeletal muscle is stretched passively, the compliant and extensible tandem Ig domains must be stretched before the stiff PEVK region of the protein is stretched (Granzier and Labeit, 2004).

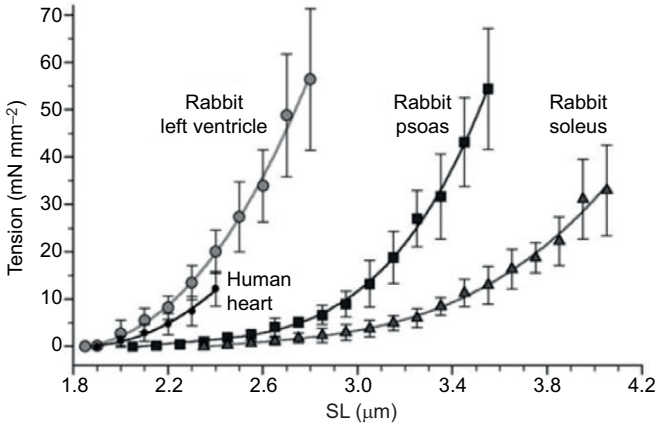

Fig. 5. Passive titin stiffness as a function of sarcomere length. Cardiac titin shows a passive increase at sarcomere lengths (SLs) well within the functional range during normal ventricular diastole. However, the skeletal muscle titin displays passive stiffness at sarcomere lengths that are outside the normal range for skeletal muscle. Reprinted from Neagoe et al. (2003).

Having both a stiff and compliant spring in series results in passive properties that, in contrast to cardiac titin, give rise to a very compliant molecule, functioning more like a bungee cord than a stiff spring. The perplexing result is that, in contrast to cardiac muscle, the passive stiffness is not tuned to normal skeletal muscle length excursions. Titin in skeletal muscle exhibits increased stiffness only at pathologically long sarcomere lengths (Neagoe et al., 2003) (Fig. 5). Thus, skeletal muscle titin has the appropriate structural characteristics to serve as the muscle spring to store and recover elastic recoil energy in skeletal muscle (Lindstedt et al., 2002), except for what seems to be an engineering flaw: its spring properties are expressed at lengths outside the physiological range of normal muscle use. One idea to reconcile this discrepancy is that some sarcomeres may be 'popped' and thus operating at very long sarcomere lengths, engaging the passive tension of titin. However, the calculated stored energy of stretched fibers was found to be far less than the observed energy storage, even when adding the passive tension of titin in popped sarcomeres to maximum contributions of tendons and cross-bridges (Linari et al., 2003).

What can explain these differences between cardiac and skeletal muscle titin? Cardiac and skeletal muscles are both striated, and they share common properties of excitation–contraction coupling and force production. However, there is one significant difference in their function: cardiac muscle never experiences isometric or eccentric contractions, it only shortens when contracting, producing ($P \times V$) work. In contrast, skeletal muscles, in particular those involved in locomotion, commonly experience isometric and lengthening contractions.

Because sufficient data about this ubiquitous muscle protein are now available (finer detail of observation), new hypotheses of muscle function are emerging. The 'winding filament hypothesis' (Nishikawa et al., 2012) is described in detail elsewhere in this issue (Nishikawa, 2016); I focus here on the distinction between the demands on these two kinds of striated muscle. What if titin is not only a passive spring (as in cardiac muscle) but also an active spring that partners with the cross-bridges in sarcomeres that undergo stretch or produce force without changing their length?

Titin as a calcium-activated clutch

What if the compliant spring (the tandem Ig domains) is dynamically altered in response to changing demand, even if

instantaneous? When a muscle is activated, is it possible that a portion of the titin molecule binds to actin, functionally eliminating the compliant Ig domain and engaging the stiff PEVK region? For this to occur, titin would have to have Ca^{2+} binding sites and would require binding to actin in the presence of Ca^{2+}. Two recent discoveries of skeletal muscle titin demand attention. First, I-band titin does bind Ca^{2+} – in fact, 12 molecules for each molecule of titin (Tatsumi et al., 2001); and second, an epitope of T2 titin binds to actin in the presence of Ca^{2+} – indeed, titin binds Ca^{2+} with a higher affinity than does troponin (Kellermayer and Granzier, 1996).

In addition, Leonard and Herzog (2010) showed that titin stiffness is much higher in active than in passive myofibrils stretched beyond overlap of the thick and thin filaments. In fact, muscle static tension increases even before cross-bridges are formed (Bagni et al., 2002, 2004). One possible explanation for this increased stiffness, as suggested by Leonard and Herzog (2010) and Nishikawa et al. (2012), is the binding of titin to actin. Although details of where along the titin molecule this may occur are not yet known, a logical candidate is the N2A region that separates the Ig (compliant) and PEVK (stiff) segments of the titin filament. Based on the existing evidence, the most parsimonious explanation is that, in the presence of Ca^{2+} (i.e. any time the muscle is active), titin binds to actin – functionally eliminating the compliant part of the molecule. By functioning as a dynamic 'clutch', this mechanism could allow the muscle to become instantaneously stiffer independent of the initial muscle length. In addition, evidence is building that titin interacts with myosin as well to modulate this dynamic shift in titin function. The details and implications of this interaction are described in detail in this issue by Nishikawa (2016). In summary, the contrasts between cardiac and skeletal muscle in their intrinsic properties provide additional evidence of how titin function can be altered in response to the nature of the muscle contraction.

Cardiac muscle never actively lengthens, it has a much stiffer titin and there is no evidence that titin stiffness changes dynamically during a normal cardiac cycle. A dominant isoform of titin found only in cardiac muscle, N2B, lacks the N2A region where the binding to actin likely occurs. In other words, cardiac titin is stiff and appears to have limited capacity for dynamic changes in stiffness *in vivo*. In contrast, skeletal muscle often experiences stretch, especially during locomotion. Two shifts in titin structure make it ideal as a potential dynamic 'clutch' that could modify muscle stiffness instantaneously, at any muscle length. If titin is a dynamic Ca^{2+}-activated spring, the result is that muscle could become instantly stiffer, and at any muscle length.

Conclusions

Our view of muscle has changed a great deal from a genetically fixed tissue specializing in producing tension, to a phenotypically plastic tissue that responds structurally and functionally to the nature of the demands placed on it. The recent focus on the properties and consequences of lengthening (eccentric) muscle contractions has provided insights into muscle function. In particular, when muscle is investigated *in vivo*, what emerges is a remarkable machine that stores and recovers energy, minimizing locomotor energy costs. Further, the high-force production coupled with the low-energy cost has suggested that lengthening contractions may be ideal for a muscle-building intervention. The observation that the cost of force production is much lower when a muscle is stretched may be best explained by an internal muscle spring. Not incorporated into the original sliding filament theory, titin is an adaptable spring within muscle that may allow muscle to respond to perturbations with nearly instantaneous changes in stiffness. Evidence for the functional role of titin is provided by contrasting the significant differences in titin structure in the context of the different demands on cardiac and skeletal muscle. Cardiac muscle is only stretched passively and it contains a very stiff titin isoform. In skeletal muscle, titin is too compliant to be a useful contributor for elastic energy storage, unless at very long sarcomere lengths. However, if bound to actin, the stiff region of the titin molecule would be engaged and titin, in active skeletal muscles, may be the key element in storage and recovery of elastic energy.

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Competing interests

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