

Is the spring quality of muscle plastic?

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Reich, T. E., S. L. Lindstedt, P. C. LaStayo, and D. J. Pierotti. Is the spring quality of muscle plastic? *Am J Physiol Regulatory Integrative Comp Physiol* 278: R1661–R1666, 2000.—During locomotion, major muscle groups are often activated cyclically. This alternate stretch-shorten pattern of activity could enable muscle to function as a spring, storing and recovering elastic recoil potential energy. Because the ability to store and recover elastic recoil energy could profoundly affect the energetics of locomotion, one might expect this to be an adaptable feature of skeletal muscle. This study tests the hypothesis that chronic eccentric (Ecc) training results in a change in the spring properties of skeletal muscle. Nine female Sprague-Dawley rats underwent chronic Ecc training for 8 wk on a motorized treadmill. The spring properties of muscle were characterized by both active and passive lengthening force productions. A single “spring constant” ($\Delta\text{force}/\Delta\text{length}$) from the passive length-tension curves was calculated for each muscle. Results from measurements on long heads of triceps brachii muscle indicate that the trained group produced significantly more passive lengthening force ($P = 0.0001$) as well as more active lengthening force ($P = 0.0001$) at all lengths of muscle stretch. In addition, the spring constants were significantly different between the Ecc (1.71 N/mm) and the control (1.31 N/mm) groups. A stiffer spring is capable of storing more energy per unit length stretched, which is of functional importance during locomotion.

eccentric contraction; muscle spring; elastic energy; titin; stiffness

DURING RUNNING LOCOMOTION, mammals behave like masses on springs, storing and reclaiming elastic recoil energy (27). The ability to reclaim elastic potential energy during the lengthening phase of the stretch-shorten pattern of muscle activity reduces the mechanical cost of the subsequent shortening contraction (2, 33). Mammals are thought to maximize elastic recoil energy by running at their natural stride frequency; i.e., during running, mammals select a stride frequency that maximizes the storage and release of elastic recoil energy (27). Activities that can change the nature of the “spring” in which this energy is stored could impact both stride frequency as well as the cost of locomotion.

Locomotion is a cyclic process with two phases per stride. Gravitational, elastic, and kinetic energy transformations occur cyclically with each stride as the body accelerates and decelerates. Either this energy dissi-

pates as heat or is stored as elastic recoil energy (24, 27). Up to half of the energy needed to accelerate the body and lift the center of mass during the shortening phase of the stride can be reclaimed from the elastic recoil energy stored in the lengthening [eccentric (Ecc)] phase of the stride (2, 27). In this way, skeletal muscle functions as a spring conserving mechanical energy.

Consequently, animals seem to select stride frequencies that maximize the performance of these springs (2, 27). If animals are made to run or hop at frequencies faster or slower than these natural frequencies, the contributions of elastic recoil energy decrease (from as much as 50 to 0%) (27). The fundamental similarity among animals (whether hoppers, gallopers, or runners) is that body mass sets the stride (or hop) frequency, which, in turn, is directly linked to the cost of locomotion.

For a muscle to function as a spring, it must contract eccentrically. When a muscle develops tension while lengthening, for example triceps while lowering one's self from a pull-up, it is performing Ecc work. In contrast, concentric muscle work occurs when a muscle develops tension while shortening. During trotting and galloping, quadrupeds experience Ecc contractions when the foot first touches the ground before a concentric contraction. The Ecc components of movement provide the deceleration forces needed for the maintenance of balance, stability, posture, and mobility (4), but also, like a spring, these lengthening contractions have the potential of providing the muscle with elastic recoil energy (2, 33).

Ecc contractions also differ in force-generating capabilities of the muscle. Skeletal muscle is capable of generating far greater tension when contracting eccentrically as opposed to concentrically or isometrically (1, 13, 17). This unique property suggests that Ecc training can potentially produce functional changes in skeletal muscle that are both qualitatively and quantitatively different from those produced by concentric exercise.

The purpose of this study is to specifically address the following question: does chronic Ecc training result in a change in the spring properties of skeletal muscle? Because the ability to store and recover elastic recoil energy could profoundly affect the energetics of locomotion, one might expect this to be an adaptable feature of skeletal muscle as are the metabolic and contractile properties of muscle (24). To test for a change, two fundamentally different measurements of stiffness were determined. One involved the measurement (in situ) of active lengthening force (dynamic stiffness) and the

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other a measurement of passive lengthening force. The active lengthening force measurement (23) was used to mimic physiological lengthening contractions that occur in locomotion. The measurement of passive lengthening force, which has a longer experimental history (12, 20, 30, 31) involving the passive stretching of muscle, may not be physiological, yet is an indicator of the muscle's passive spring properties. From results of the passive lengthening measurement, an estimate of the spring constant could be derived. This constant provided a quantitative measure of a change in the spring quality of muscle due to Ecc training.

METHODS

Ecc treadmill running. Nine female Sprague-Dawley rats, 11 wk old (posttraining body mass ranging from 165 to 280 g, mean 264 g, SE 4.7), were run downhill on a motor-driven treadmill at a speed of 16 m/min (not every rat was used for every data point for technical reasons). This speed was slow enough that the rats could not propel themselves down the treadmill but rather needed to work in an Ecc fashion to brake or decelerate. A control group, consisting of six age-matched nontrained rats (posttraining body mass 259–305 g, mean 274 g, SE 5.2) did no running. We used Ecc training as a stimulus for a change in the spring properties of skeletal muscle, because of the unique properties of Ecc training, and were not looking at the effects of different modes of training. After a 10-day familiarization period with the treadmill by running for 10 min a day (3 days), the rats ran daily for 30 min, 5 days a week for 7–9 wk. This protocol was modified from that used by Darr and Schultz (3) and Schwane and Armstrong (25). After the third week of running, the grade of the decline was increased from -26 to -36% . Delayed onset muscle soreness (DOMS), normally associated with Ecc exercise, does not occur when the Ecc load is gradually ramped (17). Positive reinforcement (raisins) was used to train the rats to run. The control rats were also given raisins once a week after being weighed. To increase the Ecc effort, the rats wore weighted vests while running (progressively increasing the weight weekly by 3% until they reached an additional 15% of their body weight) constructed of a simple Velcro design and lead clincher fishing weights that slipped over their heads and snapped under their abdomens.

Mechanical testing. At the end of the training period, the control ($n = 6$) and the experimental rats ($n = 9$) were anesthetized with pentobarbital sodium (45 mg/kg ip supplemented as required). The long head of the triceps brachii muscle was isolated from the surrounding tissue at its insertion, leaving the blood supply intact. The branches of the radial nerve leading to other triceps heads and shoulder muscles were cut. The long head of the triceps brachii was tied off with braided monofilament (30-kg test) fishing line at the most distal insertion to the olecranon process at the myotendinous junction. The compliance of the ligature was negligible relative to the muscle. A small piece of the olecranon process was chipped away from the rest and used as an anchor to prevent the knot from slipping off the myotendinous junction at high forces. This line was attached to a dual servoforce transducer (Aurora model 305B). The rat was placed in a sling, and the scapula and ulna were clamped to assure an isometric preparation. Bipolar silver electrodes attached to a Grass stimulator (model S48) were placed on the muscle for direct muscle stimulation. A bath of paraffin oil covered the muscle to prevent drying and assure a constant temperature. Bath temperature was maintained at $37 \pm 1^\circ\text{C}$

with a heating lamp and monitored with a temperature probe.

Stiffness measurements. Stimulation voltage (2-ms pulse duration) was adjusted to recruit 100% of the muscle fibers (determined by measuring maximum force), and a length/tension profile (150-ms pulse duration) was generated to determine muscle length at which maximal force production occurred (l_0). Two mechanical procedures were used to measure muscle spring qualities. The first measured active lengthening force and the second passive lengthening force.

Active lengthening force production. This procedure involved a 200-ms tetanus (supramaximal stimulation $\sim 10\%$ above voltage needed to reach maximum force, 70–100 V, at a frequency of 200 Hz). At 100 ms into the tetanus, a 0.58-mm ramp stretch (equal to $\sim 1.5\%$ resting muscle length) produced a lengthening contraction for an additional 100 ms. After the 200 ms of total stimulation, the muscle length returned to l_0 . Two minutes between tests allowed the muscle to recover. This test was repeated at five length intervals of 0.5 mm, increasing from l_0 . At each of the five points, active peak force minus maximal isometric force (P_0) (N/muscle mass) was plotted as a function of stretch (mm) [modified from Petit et al. (23)].

A Superscope (Somerville, MA) program was developed using one output channel to activate stimulation and another to move the lever 0.58 mm. A delay circuit provided a 100-ms delay between initial stimulation and lever movement. One input channel measured force production, and the other measured lever movement. Both inputs were monitored through an analog-to-digital board sampling at 1,000 Hz and dedicated microcomputer (Macintosh 7200/120).

Passive lengthening force production. To measure passive stiffness, changing the voltage input to the length servo of the lever system imposed 1-mm muscle length changes. The triceps muscles in this study were stretched in 1-mm intervals from l_0 to $l_0 + 9$ mm. Passive force production was recorded at each millimeter length interval. Because the fibers in this muscle are pennate, the 25–30% stretch of the whole muscle measured from l_0 would necessarily stretch the individual fibers more. To specifically test whether the spring properties of the muscle responded to Ecc training by becoming stiffer, a linear regression was used to estimate the "spring constant" using the formula passive force = $B_0 + B_1$ length + E, where B_1 (N/mm) is the spring constant and E is the residual. Although the passive length-tension curves were not linear, we divided the curves into two sections and ran linear regressions on each.

After the physiological measurements were taken, muscle length and circumference were measured at l_0 , and the rat was euthanized with an overdose of pentobarbital sodium. The long heads of the triceps muscles from both sides of the rat were excised. The muscles were blotted to remove excess fluid and weighed (Table 1).

Statistical analysis. Data were analyzed with a model one fixed effects repeated-measures analysis of covariance with length as the covariate. Results were considered significant if

Table 1. Weight of left triceps brachii muscle averaged across group

Control ($n=6$)		Eccentric ($n=8$)	
Wt, g	Specific wt, g/g body mass	Wt, g	Specific wt, g/g body mass
1.21 \pm 0.03	0.0044 \pm 0.0011	1.08 \pm 0.04	0.0041 \pm 0.0013

Values are means \pm SE. Weight standardized to body mass (g muscle/g body mass).

$P \leq 0.05$. A regression analysis was used to estimate the spring constant (B_1) for each animal, which was then analyzed with a t -test. Where significant differences were noted, a post hoc Student-Newman-Keuls method was used to identify all pairwise effects.

RESULTS

Active lengthening force production. Triceps from Ecc-trained animals produced 20–33% more active lengthening force than the control animals (Fig. 1). There is a significant difference in force between the two groups ($P = 0.0001$); however, there is not a significant difference in force due to the length increases between each 200-ms stimulation ($P = 0.15$). In other words, after the initial 0.58-mm stretch of the muscle, further stretching does not influence the active lengthening force production. The active force attained by the muscle at the end of the lengthening contraction was greater than the P_0 . Figure 1 shows active peak force (total force) minus P_0 (dynamic stiffness) at five different lengths. The mean dynamic stiffness for the Ecc group, i.e., at the first length interval, was 6.13 ± 0.49 (N/g muscle mass), ~25% greater than that of the control group 4.94 ± 0.47 (N/g muscle mass). The response to stretch from the Ecc group is greater than that of the control group at all five lengths.

Passive lengthening force production. Triceps from the trained group produced up to 50% more passive lengthening force than the control animals (Fig. 2). There is a significant difference in the passive force due to length ($P < 0.0001$) as well as due to group ($P < 0.0001$). Regardless of the length of stretch, the Ecc group ($n = 8$) produced greater passive force than the control group ($n = 6$). Figure 2 shows passive length-tension curves. To test whether the muscle responded adaptively to the physiological demands of chronic Ecc

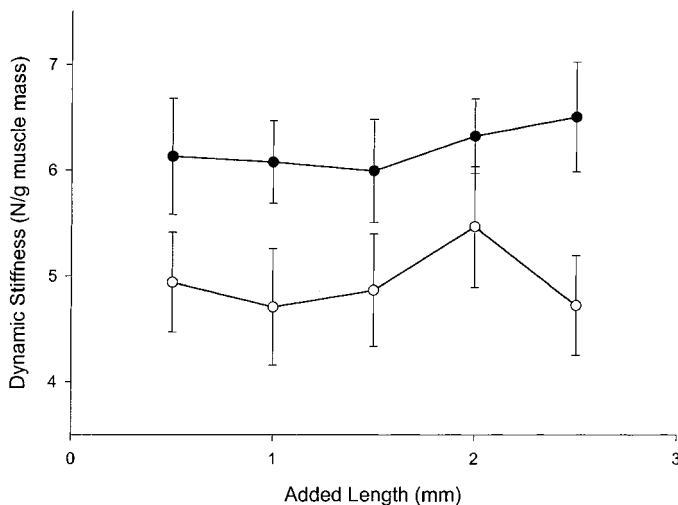


Fig. 1. Each data point represents the averaged active peak force minus maximal isometric force for group comparisons at 5 lengths. Top line (●) is the eccentric (Ecc) group data ($n = 9$), and the bottom line (○) is the control group data ($n = 5$). Force is normalized to muscle mass, and length is measured in mm. There is a significant difference in force between the 2 groups ($P = 0.0001$); however, there is not a significant difference in force due to stretch beyond the initial 0.58 mm stretch ($P = 0.1522$). Error bars are SE. Length = 0 is l_0 .

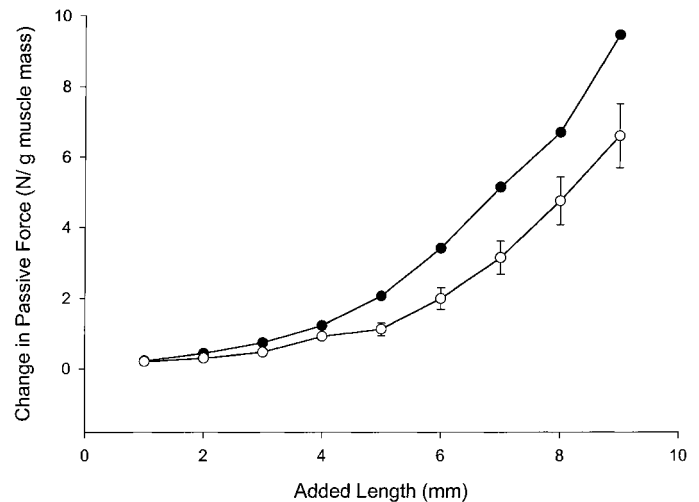


Fig. 2. Length-tension curves comparing Ecc-trained vs. the untrained groups. ● represent Ecc group data ($n = 8$); ○ are the control group data ($n = 6$). Error bars are equal to SE. Force is standardized to muscle mass, and length is measured in mm. The muscles were passively stretched in 1-mm increments beginning at l_0 and ending at 9 mm. There is a significant difference in the passive force at all lengths ($P < 0.0001$), as well as due to group ($P < 0.0001$).

training by becoming stiffer, we divided the curves into two sections and ran linear regressions on each. There is no statistical significance between the slopes of the first half of the curves (lengths 1–4). There is, however, a significant 30% difference ($P = 0.038$) between the spring constant for Ecc group (1.71 N/mm) and that of the control group (1.31 N/mm) for the second half of the curves (lengths 5–9). The Ecc group produced more force than the control group for a fixed amount of length change at high strains.

DISCUSSION

The purpose of this study was to examine the impact of chronic Ecc training on the spring properties of skeletal muscle. After the training period, two measurements of muscle spring stiffness were quantified.

Our results indicate that similar to other structural and functional properties of skeletal muscle, the muscles' spring qualities are plastic. At this point, it is unclear whether the mechanism of change involves an isoform change (qualitative shift) or a change in abundance (quantitative shift); however, the mechanism of change is the next question to be addressed.

Every spring-mass system has a natural frequency at which the system will oscillate. Ontogenetically, a change in spring properties occurs with a change in body size to maximize the return of elastic energy during locomotion. Apparently, increases in physiological demands such as chronic Ecc training can alter these size-dependent constraints as well. On the basis of the comparisons of the two spring constants, muscle adapts to the physiological demands of chronic Ecc training by becoming stiffer (increased slope of the passive length-tension curves) and by producing more force per unit length change when contracting eccentrically. This change in the spring system may serve as a

mechanism protecting the muscle from possible damage due to Ecc training.

The difference in active lengthening force production between the two groups is not due to an increase in muscle size (Table 1), as muscle mass normalized to body mass is not significantly different between the two groups. That maximal P_o is not significantly different between groups (Table 2) indicates that the trained rats did not get isometrically stronger. However, force with active lengthening is significantly different between the groups, indicating that the stiffness or spring quality of the muscle has indeed changed.

Because of its importance in locomotion, it may be expected that this characteristic of muscle is adaptable, capable of responding to shifts in demand, just as are the contractile and metabolic properties of muscle. Indeed the results of this study demonstrate that this apparently "tuned spring" within skeletal muscle is indeed plastic. It is still unclear which structure or structures within the muscle complex changed. At this point, we can speculate that a combination of three structures within the myotendinous system may be involved in the increase in force production: 1) tendon, 2) collagen, and 3) cytoskeletal proteins including titin.

Tendon. Because historically tendon was thought to be the primary source of passive tension, the production of passive tension and storage and release of elastic recoil energy in skeletal muscle was considered negligible (5, 33). However, with the growing understanding of the cytoskeletal proteins within the muscle cell, tendon is no longer considered the predominant structure responsible for the storage of elastic energy (20, 33). In this study, tendon could not have significantly contributed to the measured spring properties because the tendinous material was excised before testing.

Collagen. Magid and Law (20), using passive stretch to compare the resting tension of intact single frog muscle fibers to the resting tension of skinned single muscle fibers, found that the stiffness of the skinned and unskinned fibers was identical. They first demonstrated that the structure responsible for producing passive tension, up to sarcomere lengths of 3.8 μm , resides within the fiber and is not significantly affected by material in the extracellular matrix. Therefore, they concluded that skeletal muscle extracellular collagen is not responsible for passive tension production.

We used a whole muscle preparation in this study and, therefore, cannot completely exclude the contributions of the extracellular matrix based on the results of Magid and Law's work (20). However, their study shows that some structure within the muscle fiber itself is responsible for passive tension development. Han et al. (8) recently showed that the gene expression of collagen types I, III, and IV increases due to muscle damage after downhill running. We feel we eliminated muscle damage in our study by progressively increasing the duration, work rate, and workload of the downhill running (17). Having shown that the elastic properties of muscle are plastic, our next question is a mechanistic one and we are focusing on the contributions of the cytoskeletal protein titin, although we are not negating the possible contributions of collagen.

Cytoskeletal proteins. The intrasarcomeric (titin and nebulin) or extrasarcomeric (intermediate filaments, focal adhesions, and dystrophin) cytoskeletal proteins are involved in force transmission (22). Force is generated via the interaction of actin and myosin and is transmitted to the Z-disc by the titin molecule. The intermediate filaments coupled with focal adhesions transmit force laterally between myofibrils. Other structures transmit force to the connective tissue matrix and finally to the tendon (22). It is apparently beneficial to have a mechanically redundant system with multiple pathways of force transmission via a number of filament systems. However, the question remains which, if any, proteins within this system may have changed in response to the Ecc load to produce the functional changes seen in this study?

The location and molecular properties of titin suggest that passive force, also referred to as resting tension, arises due to the stretching of the elastic filaments of titin (9, 10, 12, 14, 16, 18, 28). This suggestion has been supported after the degradation of titin with enzymatic digestion and ionization radiation resulted in a decrease in tension exerted by the resting skinned muscle fiber (10, 22). It was concluded that the elastic properties of titin are responsible for the production of passive tension, as well as the positional stability of myosin filaments at the center of the sarcomere during activation (11, 12, 22, 29, 32).

The same pattern of passive tension development was observed in this study using the entire muscle as is

Table 2. P_o , active peak force, and dynamic stiffness standardized to muscle weight and averaged across group

	Length 1	Length 2	Length 3	Length 4	Length 5
Control					
P_o	13.13 \pm 0.40	14.10 \pm 0.67	13.87 \pm 0.59	13.92 \pm 0.69	13.75 \pm 0.58
Peak force	18.07 \pm 0.60	18.81 \pm 0.46	18.73 \pm 0.69	19.38 \pm 0.86	18.47 \pm 0.37
Eccentric					
P_o	14.10 \pm 0.75	14.76 \pm 0.87	14.83 \pm 0.96	14.82 \pm 0.98	15.01 \pm 1.2
Peak force	20.23 \pm 1.0	20.83 \pm 1.0	20.82 \pm 1.0	21.12 \pm 1.2	21.51 \pm 1.5
Dynamic stiffness (active peak force minus P_o)					
Control	4.94 \pm 0.47	4.71 \pm 0.55	4.81 \pm 0.53	5.46 \pm 0.57	4.72 \pm 0.47
Eccentric	6.13 \pm 0.49	6.07 \pm 0.40	5.99 \pm 0.49	6.30 \pm 0.32	6.50 \pm 0.52

Values are means \pm SE in N/g muscle mass. Maximal isometric force (P_o) is force produced at each interval of stretch (0.5-mm intervals, length 1 = l_o) before 0.58-mm stretch with activation is imposed. Peak force is force produced during last 100-ms stimulation with 0.58-mm stretch. Dynamic stiffness is active peak force minus P_o .

seen in isolated titin (12, 19, 20, 22); this is not surprising, because titin is the primary structure responsible for the production of passive tension produced during the passive lengthening of the muscle fiber (6, 7, 15, 19, 21, 30). Perhaps titin also contributes to the production of active tension during Ecc work, when the muscle actively lengthens to resist an external load (33). Titin may contribute by enhancing elastic energy storage and release and by maintaining sarcomere alignment, ensuring efficient muscle contraction (33). Furthermore, the expression of different titin isoforms could adjust the spring properties of the fiber to the physiological demands placed on the muscle to best maintain sarcomere structure (15, 19, 26). A stiffer, shorter titin, therefore, may explain the greater passive as well as active lengthening force-producing capabilities of the Ecc-trained rats. However, this remains to be tested.

In summary, due to the importance of elastic recoil energy in locomotion, one may expect that this characteristic of muscle is adaptable, capable of responding to shifts in demand just as are the contractile and metabolic properties of muscle. Although body mass determines an animal's "natural" frequency, the results of this study show that the spring properties of muscle are indeed adaptable and increase in physiological demands such as chronic Ecc training can alter the size-dependent constraints. An individual unaccustomed to hiking downhill will experience DOMS. However, after several hikes down, that same individual experiences little or no discomfort. The question now is which structure within the muscle is adapting, allowing for the functional changes we have seen in this study. We believe that the giant cytoskeletal protein titin may adjust the spring properties of the fiber by expressing different isoforms to best withstand changes in physiological demand. To determine whether titin is involved, RT-PCR is being used to identify potential splice variants in trained versus untrained titin.

Perspectives

The purpose of this study was to investigate whether the spring properties of muscle are plastic. Because of the importance of elastic recoil energy in locomotion, one may expect that this characteristic of muscle is adaptable. During ontogeny, mammals' stride frequencies change as a predictable function of body size. Shifts in muscle spring properties would allow for ontogenetic "tuning" of the spring to maximize locomotor efficiency. Differences in the spring properties may also accompany sexual dimorphism. In addition, apparently increases in physiological demands, such as chronic Ecc muscle use, likewise change the spring properties of muscle. Perhaps this plasticity is due to the differential expression of one molecule. Different titin isoforms have been identified (15, 19, 26); perhaps it is the differential expression of these isoforms which dictates the spring properties of skeletal muscle, for example, ontogenetically, due to sexual dimorphism and due to increases in physiological demands placed on the muscle.

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