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The magnitude of muscle strain does not influence serial sarcomere number adaptations following eccentric exercise

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Abstract It is generally accepted that eccentric exercise, when performed by a muscle that is unaccustomed to that type of contraction, results in a delayed onset of muscle soreness (DOMS). A prolonged exposure to eccentric exercise leads to the disappearance of the signs and symptoms associated with DOMS, which has been referred to as the repeated bout effect (RBE). Although the mechanisms underlying the RBE remain unclear, several mechanisms have been proposed, including the serial sarcomere number addition following exercise induced muscle damage. In the traditional DOMS and RBE protocols, muscle injury has been treated as a global parameter, with muscle force and strain assumed to be uniform throughout the muscle. To assess the effects of muscle-tendon unit strain, fiber strain, torque and injury on serial sarcomere number adaptations, three groups of New Zealand White (NZW) rabbits were subjected to chronic repetitive eccentric exercise bouts of the ankle dorsiflexors for 6 weeks. These eccentric exercise protocols consisted of identical muscle tendon unit (MTU) strain, but other mechanical factors were systematically altered. Following chronic eccentric exercise, serial sarcomere number adaptations were not identical between the three eccentric exercise protocols, and serial sarcomere number adaptations were not uniform across all regions of the muscle. Peak torque and relaxation fiber strain were the best predictors of serial sarcomere number across all three protocols. Therefore, MTU strain does not appear to be the primary cause for sarcomerogenesis, and differential adaptations within the muscle may be explained by the nonuniform architecture of the muscle, resulting in differential local fiber strains.

Keywords Sarcomerogenesis · Force-length relationship · Muscle injury · Repeated bout effect · Delayed onset muscle soreness

Introduction

It is generally accepted that eccentric exercise results in pain, soreness and disability when performed by a muscle that is unaccustomed to that type of contraction [25, 29, 46]. This pain that typically follows eccentric exercise has been termed delayed onset muscle soreness (DOMS) [25]. It is accepted that prolonged exposure to eccentric exercise reduces the signs and symptoms of DOMS, and ultimately leads to their disappearance [34]. This protective effect of eccentric exercise has been referred to as the repeated bout effect (RBE) [39]. Although there have been conflicting reports as to the cause of DOMS, the fact that an acute bout of eccentric exercise results in muscle injury is generally accepted, but the mechanisms underlying the RBE remain unclear.

The RBE has been observed since the turn of the century [16], and has been studied extensively [4, 14, 15, 32–35, 44]. However, although much information has been gleaned through years of experimental research and anecdotal evidence, the exact mechanisms remain elusive. Because eccentric exercise results in muscle injury, and skeletal muscle has an amazing ability to regenerate, repair, and adapt, an injury dependent mechanism has been proposed [42]. This cellular mechanism of adaptation is intertwined with a mechanism of muscle injury associated with eccentric exercise that depends on the number of sarcomeres arranged in series within the muscle fibers [43]. Thus, if a muscle has too few sarcomeres arranged in series, the fibers are more likely to become damaged, and they adapt through sarcomerogenesis (increasing the number of sarcomeres in series) to prevent such disruption in the future [42]. Such an injury dependent mechanism is appealing as it accounts for muscle injury, soreness, and adaptation all at once.

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Although the damage dependent model of sarcomerogenesis is attractive, most models have been explored using static stimuli, such as passive static stretch or shortening [13, 28, 48–50, 52, 54–56, 58, 59]. These tests have proven to be effective in producing rapid changes in serial sarcomere numbers within the skeletal muscle. Unfortunately, few studies have been aimed at assessing the effects of dynamic stimuli on serial sarcomere number within the skeletal muscle. Although significant alterations in serial sarcomere numbers have been shown by altering muscle excursion [5, 6, 20, 21], only three studies have looked at the effects of eccentric exercise on serial sarcomere number directly.

These latter studies utilized either an unconstrained in vivo protocol (downhill walking in rats [30, 31]) or a constrained in vivo approach (controlled eccentric contractions of rabbit dorsiflexor muscles [19]). In all the three studies, serial sarcomere numbers increased following exercise, but the mechanical stimuli that may account for these adaptations remain unknown. Interestingly, muscle injury in those protocols, as well as all traditional DOMS protocols, has been treated as a global parameter influencing muscle adaptation, with muscle force and strain having been assumed to be uniformly distributed throughout the muscle. However, there is no evidence that muscle architecture and the injury sustained following eccentric exercise is uniform across the muscle. In fact, in at least one previous study, a differential adaptation within two regions of the same muscle following eccentric exercise has been observed, but no explanation was provided for this observation [19]. Thus, the stresses and strains within these muscles remain unknown.

Recently, we measured torque and fiber lengths during repeated stretch-shortening cycles in the NZW rabbit tibialis anterior [10] using a constrained in vivo protocol. By altering the timing of activation relative to muscle stretch and the initial muscle tendon unit (MTU) length from which the stretch was started, we showed that fiber strains, torque magnitudes, and muscle injury varied, even though the strain applied to the MTU was constant [8]. The ability to directly measure muscle torque and fiber strains provides valuable insight into the possible stimuli for serial sarcomere number adaptation following chronic eccentric exercise. Therefore, the aim of this study was to test two hypotheses: first, MTU strain is the primary factor to produce sarcomerogenesis, and therefore serial sarcomere adaptations are identical following exercise protocols with identical MTU strain magnitude; second, serial sarcomere number adaptations are uniform across all regions of the muscle. By measuring the magnitudes of fiber strain, joint torque production, and muscle injury for different eccentric exercise protocols, we hope to gain further insight into the relationships between these mechanical variables and serial sarcomere number adaptations.

Methods

In order to achieve the goals of this study, one acute exercise protocol and one chronic exercise protocol were used. Within each protocol, the dorsiflexor muscles of three groups of NZW rabbits were subjected to eccentric exercise protocols consisting of a constant MTU strain, but varying starting MTU lengths and activation times. The acute exercise groups were designed to provide measures of mechanical variables during eccentric exercise. The chronic exercise groups were used to measure cellular adaptations in the tibialis anterior following chronic exercise.

Acute protocol measurement of mechanical variables

The acute protocol was designed to directly measure fiber strain, joint torque, and muscle injury. All methods have been previously reported in detail [8]. Briefly, rabbits were divided into three groups, based on the starting muscle length and the timing of muscle activation. Group one (SOS, $n=11$ TA) consisted of Stimulation at the Onset of stretch (plantar flexion), with lengthening contractions starting at a Short muscle length (70° tibiotarsal joint angle). Group two (SPS, $n=7$ TA) consisted of Stimulation Preceding stretch by 100 ms with lengthening contractions starting at a Short muscle length (70° tibiotarsal joint angle). Group three (SPL, $n=6$ TA) consisted of Stimulation Preceding stretch by 100 ms with lengthening contractions starting at a Long muscle length (95° tibiotarsal joint angle).

Chronic protocol

The chronic protocol was designed to subject the tibialis anterior of the NZW rabbit to identical mechanical stimuli as the acute protocol, but for 18 exercise bouts in total. Twenty-one skeletally mature female NZW rabbits (5.1 ± 0.5 kg, Riemens, St. Agatha, ON, Canada) were randomly selected and assigned to one of the three exercise groups corresponding to the acute protocols described above. For the chronic exercise protocol, the three groups were denoted with the subscript 'c': SOS_c ($n=7$), SPS_c ($n=8$), or SPL_c ($n=6$) to differentiate them from the acute protocol.

Surgical procedure

Seven days preceding, as well as on the day of surgery, each rabbit received a 25 mg/kg subcutaneous injection of Micotil (tilmicosin, 300 mg/kg, Provel Inc., Guelph, ON, Canada) to prevent pasteurellosis during the experimental protocol [36]. The rabbits were tranquilized with 0.18 ml Acevet (acepromazine, 25 mg/ml,

Vétoquiol, N.A. Inc., Lavaltrie, QC, Canada), and held under anesthesia with 1.5% isoflurane, 0.6 l/min N₂O, and 0.8 l/min O₂. An incision was made on the posterior aspect of the left and right hindlimbs, anterior to the sciatic vein, and the biceps femoris and semimembranosus were separated exposing the peroneal nerve. A custom-made nerve cuff electrode was secured around the left peroneal nerve and connected to a homemade interface [22]. The interface was routed subcutaneously from the hindlimb incision to the lumbar spine, where another incision was made, and the interface sutured to the erector spinae muscles of the rabbit. A second nerve cuff was also implanted on the right peroneal nerve but it was not used. Once the cuffs and interface were implanted, the incisions were closed and the rabbits were allowed to recover. Each rabbit received 0.25 ml of torbugesic (Ayerst Veterinary Laboratories, Montreal, QC, Canada) for pain modulation immediately after surgery and every 6 h thereafter for 24 h. All rabbits responded well to the surgery, moving freely throughout their cages. All exercise protocols began within 9 days following surgery.

Eccentric exercise protocol

Leads from surgically implanted nerve cuffs of the acute exercise group rabbits (*SOS*, *SPS*, and *SPL*) were directly attached to a stimulator (Grass S8800, Astro-Med Inc., Longueuil, QC, Canada). For rabbits in the chronic exercise groups (*SOS_c*, *SPS_c*, *SPL_c*), an external connection was made using two silicone insulated ten strand cables (Crooner Wire Company, Chatsworth, CA, USA) threaded through two short beveled 22 gauge needles (Becton-Dickinson and Company, Franklin Lakes, NJ, USA). The ends of the wires were bared and barbed, and the needles were inserted through the skin of the rabbit into the two wells of the interface. The needles were then removed from the interface, leaving the wire connected [22]. After the nerve cuff was connected to the stimulator, the α -motoneuron threshold was determined (pulse duration = 0.1 ms, frequency = 150 Hz, train duration = 500 ms).

All rabbits were anaesthetized and placed supine in a stereotaxic frame with the knee joint at 90°. The foot was strapped to a servo-motor foot plate (Parker Hannifin Corporation, Irwin, PA, USA) and ankle movement was controlled via Motion Planner software (Compumotor, Rohnert Park, CA, USA). The medial condyle of the tibia and the medial malleolus were marked, and using a small plastic goniometer, the tibiotarsal joint angle was set at 90° (increased tibiotarsal joint angle = increased plantar flexion), which served as the reference angle for the remainder of the experiment.

Immediately preceding the first exercise bout, an isometric torque–joint angle relationship was determined by supramaximally stimulating (3 \times α -motoneu-

ron threshold voltage, pulse duration = 0.1 ms, frequency = 150 Hz, train duration = 2000 ms) the dorsiflexor muscles, beginning at a tibiotarsal angle of 55° and progressing in 5° increments to 155° (i.e., 21 measurements). The foot was returned to a dorsiflexed position (55° tibiotarsal angle) for 2 min of rest between contractions. Subsequently, cyclic lengthening contractions were performed from a tibiotarsal angle of 70–105° of plantar flexion at 70° s⁻¹ for the *SOS*, *SPS*, *SOS_c*, and *SPS_c* groups, and from 95 to 145° of plantar flexion at 100° s⁻¹ for the *SPL* and *SPL_c* groups. All the three exercise protocols resulted in an MTU strain of 5% for all the groups. The protocol consisted of 5 sets of 10 cyclic lengthening contractions with a 2 min rest between sets. For the acute groups, a post-exercise torque–angle relationship was obtained following the single exercise bout, and then the rabbits were euthanized. For the chronic exercise groups, each rabbit was exercised 3 times per week for 6 weeks with at least 48 h of rest between bouts. Afterwards, the rabbits were allowed to recover and were returned to their cages. One week following the last exercise bout (bout no. 18), another torque–joint angle relationship was obtained for the 21 tibiotarsal joint angles (55–155° in 5° increments) for direct comparison to the pre-exercise values. The 1 week rest between the exercise and the measurement of the torque–joint angle relationship assured full recovery of the muscles.

Data collection

The joint torque was measured from the strain gauges placed on the cam between the servomotor and the footplate. The output signal was routed through a strain gauge amplifier/conditioner (Vishay 2100, Vishay Micro-Measurements, Raleigh, NC, USA. Gain 8.44 \times 100; Excitation 10 V dc; Low pass filter 20 Hz) and sampled at 250 Hz with the WinDaq data acquisition software (Dataq Instruments, Akron, OH, USA). In addition, for the acute groups (*SOS*, *SPS*, and *SPL*), fiber length measurement data were collected via Sonosoft data acquisition systems (Sonometrics Corporation, London, ON, Canada) at 498 samples per second.

Tissue was harvested from each rabbit in the chronic exercise group 48 h following the second torque–joint angle relationship measurement. Rabbits were tranquilized using a Ketalean–Xylazine combination (50 mg/kg Ketalean, Bimeda-MTC, Cambridge, ON, Canada; 10 mg/kg Xylazine, Boehringer Ingelheim, Burlington, ON, Canada), and subsequently euthanized by a 2.5 ml injection of Euthanyl (pentobarbital sodium, MTC Inc., Cambridge, ON, Canada) in the lateral ear vein. Both hindlimbs were removed, skinned, and pinned at 90° knee and tibiotarsal joint angle. The hindlimbs were fixed in 10% neutral-buffered formalin for at least 6 weeks prior to sarcomere and fascicle length measurements.

Data analysis

Tissue analysis procedure

After fixation, the tibialis anterior muscles were carefully removed from the tibia, blotted dry, and weighed. Using directly measured fiber length (at fixation angle of 90° joint angle) and muscle mass, the physiological cross sectional area (PCSA) was calculated from the equation: $PCSA \text{ (cm}^2\text{)} = \text{muscle mass (g)/fiber length (cm)} \times \text{muscle density of } 1.0564 \text{ g/cm}^3$ [37]. Muscles were then cut into medial, lateral, and central regions, and placed in 30% nitric acid for 10 h. Six fascicles were teased from four regions of each muscle (medial, lateral, central-superficial, central-deep), containing approximately ten fibers per fascicle. The specimens were placed on slides with glycerol, and their lengths were measured with video analysis software (Matrox Systems Incorporated, Dorval, QC, Canada). Sarcomere lengths were measured at six points along the length of the fascicle by laser diffraction [19, 20, 27, 31] (15 mW Helium–Neon laser, beam diameter 0.8 mm, wavelength 633 nm, Meredith Instruments, Glendale, AZ, USA). Sarcomere number per fascicle was determined by dividing the fascicle length by the average sarcomere length. The sarcomere number represents the average number of sarcomeres per fascicle.

Torque joint–angle relationship analysis

Peak torque values were plotted for all 21 tibiotarsal joint angles. Torque values for each individual rabbit were plotted pre-exercise and post-exercise. To calculate the angle of peak torque occurrence for each rabbit, all torque values >75% of the absolute peak torque were normalized, fit with a second-order polynomial, and peak torque was calculated as the peak value of the polynomial approximation [7, 17]. Individual shifts in the torque–joint angle relationships were calculated by comparing the angle of peak isometric torque post-exercise to the angle of peak isometric torque production pre-exercise. The mean shift in peak torque production was calculated for each chronic exercise group (SOS_c , SPS_c , SPL_c).

Statistical analyses

All statistical analyses were performed using SPSS Version 13.0 (SPSS Incorporated, Chicago, IL, USA). Serial sarcomere number comparisons between exercised and control limbs were analyzed using Student's paired *t*-tests for each region of the muscle. For comparison between exercise groups, the magnitude of the change in the serial sarcomere number following exercise was first expressed as a percentage change for each rabbit. Then, the differences between the exercise groups were analyzed using one-way analysis of variance with a least squared difference post hoc test. All torque values were

analyzed using a Kruskal–Wallis chi-squared test. For comparison of acute variables to chronic measurements, multiple linear regressions were used. For all parameters measured, mean \pm SE are reported. Statistical significance was set at $p < 0.05$.

Results

Acute protocol

Fiber strain, torque, and injury variables

Fiber strains have been previously defined [10], and those definitions are also used for this study. Fiber strains, joint torque, and muscle injury values for all three acute exercise groups have been presented previously [8]. Here, we calculated the mean value for all previously reported variables for each protocol (Table 1), to assess the potential relationship between those variables and the adaptive changes we observed in the present 6 weeks chronic protocol. By performing this statistical analysis, we assume that the mechanical variables measured in the rabbits of the acute protocol are similar to those experienced by the rabbits of the chronic protocol.

Chronic protocol

Pre-exercise architectural measures

The tibialis anterior of the right hindlimbs were used as normal control muscles. Assuming that the structure of the tibialis anterior does not differ between hindlimbs of each rabbit prior to exercise, the control muscles can also illustrate the architectural properties of the muscle

Table 1 Means \pm SE of measured mechanical variables during the corresponding acute eccentric exercise protocols

Variable	Exercise protocol		
	<i>SOS</i>	<i>SPS</i>	<i>SPL</i>
MTU strain (%)	5.0 \pm 0.2	5.0 \pm 0.5	5.2 \pm 0.7
Active shortening (%)	−6.1 \pm 0.3	−8.2 \pm 0.2	−6.1 \pm 0.2
Active strain (%)	10.2 \pm 0.1	11.9 \pm 0.1	15.6 \pm 0.1
Maximum strain (%)	12.9 \pm 0.1	20.6 \pm 0.2	24.6 \pm 0.2
Relaxation strain (%)	2.5 \pm 0.2	7.7 \pm 0.2	7.8 \pm 0.2
Peak torque (Nm)	0.6 \pm 0.02	0.7 \pm 0.03	0.7 \pm 0.03
Isometric torque reduction (%)	−35.9 \pm 2.1	−40.1 \pm 2.2	−69.3 \pm 3.0
Eccentric torque reduction (%)	−26.9 \pm 1.8	−37.3 \pm 2.7	−50.2 \pm 3.8
Force-length relation shift (°)	7.7 \pm 1.9	7.9 \pm 1.9	10.9 \pm 1.0

SOS stimulation at onset of plantar flexion at short muscle lengths, *SPS* stimulation preceding plantar flexion at short muscle lengths, *SPL* stimulation preceding plantar flexion at long muscle lengths. For description of all variables (see Ref [8])

prior to exercise. At a tibiotarsal joint angle of 90°, fiber and sarcomere lengths between the four regions of all control muscles were nonuniform. Fiber lengths in the lateral and central-superficial regions were the longest (48.0 ± 0.5 and 47.4 ± 0.5 mm, respectively) and were not significantly different from each other ($p = 0.371$). However, the fibers of the medial and central-deep regions were significantly shorter (44.7 ± 0.5 and 43.6 ± 0.3 mm, respectively) than the fibers of the central-superficial and lateral regions ($p < 0.001$), but were not significantly different from each other ($p = 0.258$).

Average sarcomere lengths also varied across regions of the control muscles. The sarcomere lengths within the fibers of the medial and central-deep regions (short fibers) were virtually identical (2.17 ± 0.01 μm compared to 2.17 ± 0.01 μm , $p = 0.8732$). However, between the regions comprising the long fiber lengths (central-superficial and lateral regions), the average sarcomere lengths differed. Average sarcomere length in the central-superficial region (2.15 ± 0.01 μm) was not significantly different from the sarcomere lengths in the short fibers (medial and central-deep regions $p > 0.05$). The average sarcomere length in the lateral region (2.21 ± 0.01 μm) was significantly longer compared to all other regions ($p < 0.05$).

Adaptations in serial sarcomere number

Over the course of the 6 weeks of eccentric exercise training protocol, the weights of the rabbits did not change significantly (5.13 ± 0.5 kg compared to 5.17 ± 0.4 kg, $p = 0.321$). The PCSA of the control limbs was not significantly different between the three exercise groups (1.04 ± 0.05 cm^2 SOS_c group; 1.01 ± 0.05 cm^2

SPS_c group; 1.06 ± 0.04 cm^2 SPL_c group; $p = 0.774$). Following the exercise protocols, there were significant training effects on the tibialis anterior muscles, as the PCSA of the trained muscle was significantly greater compared to the control muscle in all three groups ($p < 0.05$). Between exercise groups, the PCSA following the six-week-exercise protocol was also significantly different (1.3 ± 0.05 cm^2 SOS_c group; 1.2 ± 0.05 cm^2 SPS_c group; 1.5 ± 0.02 cm^2 SPL_c group; $p = 0.003$). Post hoc analysis revealed that chronic exercise at long muscle lengths resulted in a greater increase in PCSA of the TA ($p < 0.05$) when compared to the increase in PCSA resulting from exercise at short muscle lengths. At short muscle lengths, however, altering the timing of activation did not significantly influence the change in PCSA ($p > 0.05$).

Following chronic eccentric exercise, there were significant serial sarcomere number adaptations in the exercised muscles compared to the muscles of the contralateral control limbs in all the three protocols. Within the four selected regions of the exercised TA and between the three protocols, serial sarcomere number adaptations varied. These values are compiled in Table 2. At short muscle lengths, the medial aspect of the muscle experienced a significant loss of serial sarcomere number in the SOS_c group ($p < 0.001$), but there was no significant change in the SPS_c group ($p = 0.075$) following exercise. Chronic exercise at the long muscle length (SPL_c) resulted in a significant increase in serial sarcomere number in the medial portion of the TA compared to control ($p = 0.005$, Table 2). Between groups, the significant increase in serial sarcomere number in the medial portion of the TA ($+3.0 \pm 1.4\%$, Table 2) following eccentric exercise at long lengths (SPL_c) was significantly different from the change in the SOS_c group ($p = 0.012$) but not the

Table 2 Serial sarcomere number in the four regions of the tibialis anterior muscle for left (exercised) and right (control) hindlimbs for all the three chronic exercise protocols

Protocol	Serial sarcomere number		Change (%)	P-value
	Control	Exercised		
Central-superficial region				
SOS_c	22377 ± 401	23084 ± 425	2.7 ± 1.4	0.015
SPS_c	22164 ± 346	23813 ± 335	7.6 ± 0.9	< 0.001
SPL_c	21420 ± 310	22744 ± 273	6.4 ± 1.1	< 0.001
Central-deep region				
SOS_c	20442 ± 272	20101 ± 328	-2.0 ± 1.4	0.712
SPS_c	20494 ± 270	19706 ± 301	-3.3 ± 2.3	< 0.001
SPL_c	19001 ± 217	18113 ± 252	-4.7 ± 2.3	< 0.001
Medial region				
SOS_c	20738 ± 397	19573 ± 261	-5.0 ± 2.8	< 0.001
SPS_c	20524 ± 304	20071 ± 312	-2.4 ± 1.3	0.075
SPL_c	20327 ± 175	20995 ± 250	3.0 ± 1.4	0.005
Lateral region				
SOS_c	21601 ± 402	21088 ± 362	-2.2 ± 2.1	0.015
SPS_c	22091 ± 311	22250 ± 391	0.7 ± 1.3	0.465
SPL_c	21270 ± 232	21137 ± 249	-0.6 ± 1.4	0.743

SOS_c stimulation at the onset of plantar flexion at short muscle lengths, SPS_c stimulation preceding plantar flexion at short muscle lengths, SPL_c stimulation preceding plantar flexion at long muscle lengths. Values are mean \pm SE

SPS_c group ($p=0.066$). Differences in serial sarcomere number adaptations in the medial TA between both groups that exercised at short lengths were not significantly different ($p=0.351$, Fig. 1a).

In the lateral aspect of the TA, chronic eccentric exercise also resulted in a significant decrease in serial sarcomere number in the SOS_c group ($p=0.015$, Table 2), but no significant change in the SPS_c group ($p=0.465$) or the SPL_c group ($p=0.743$, Fig. 1b). Between groups, the serial sarcomere number changes (Table 2) were not significantly different regardless of the activation timing or starting length of the muscle prior to exercise ($p=0.456$, Fig. 1b).

Serial sarcomere numbers within the central-superficial region (Table 2) following chronic eccentric exercise were significantly different compared to controls for all three exercise groups (Fig. 1c). Specifically, there were significant serial sarcomere number increases in the SOS_c group ($p=0.015$), the SPS_c group ($p<0.001$), and the SPL_c group ($p<0.001$). Between the groups (Table 3), pre-activation at short (SPS_c group) as well as long (SPL_c group) muscle length resulted in serial sarcomere number adaptations that were significantly greater than in the SOS_c group ($p=0.006$ and 0.041 ,

respectively). However, the differences in adaptation between the two pre-activation groups (SPS_c group and the SPL_c group) were not significant ($p=0.482$, Fig. 1c).

In the central-deep region of the TA, sarcomere numbers decreased (Table 2) following chronic eccentric contractions in all three protocols (Fig. 1d). In the SOS_c group, the loss of serial sarcomeres in the central-deep portion of the TA was not significant when compared to the TA of the contralateral control limb ($p=0.712$). However, the loss of serial sarcomeres following eccentric exercise was significant in both the SPS_c group ($p<0.001$) and the SPL_c group ($p<0.001$), which were pre-activated prior to the stretch. However, between the three exercise groups (Table 2), there were no significant differences ($p>0.05$).

Adaptations in torque production

Following chronic eccentric exercise in all three protocols, the peak of the torque–joint angle relationship was shifted to the right. This shift indicated that the peak joint torque was produced at longer muscle lengths (Figs. 2, 3, 4), and the magnitude of the shifts was sig-

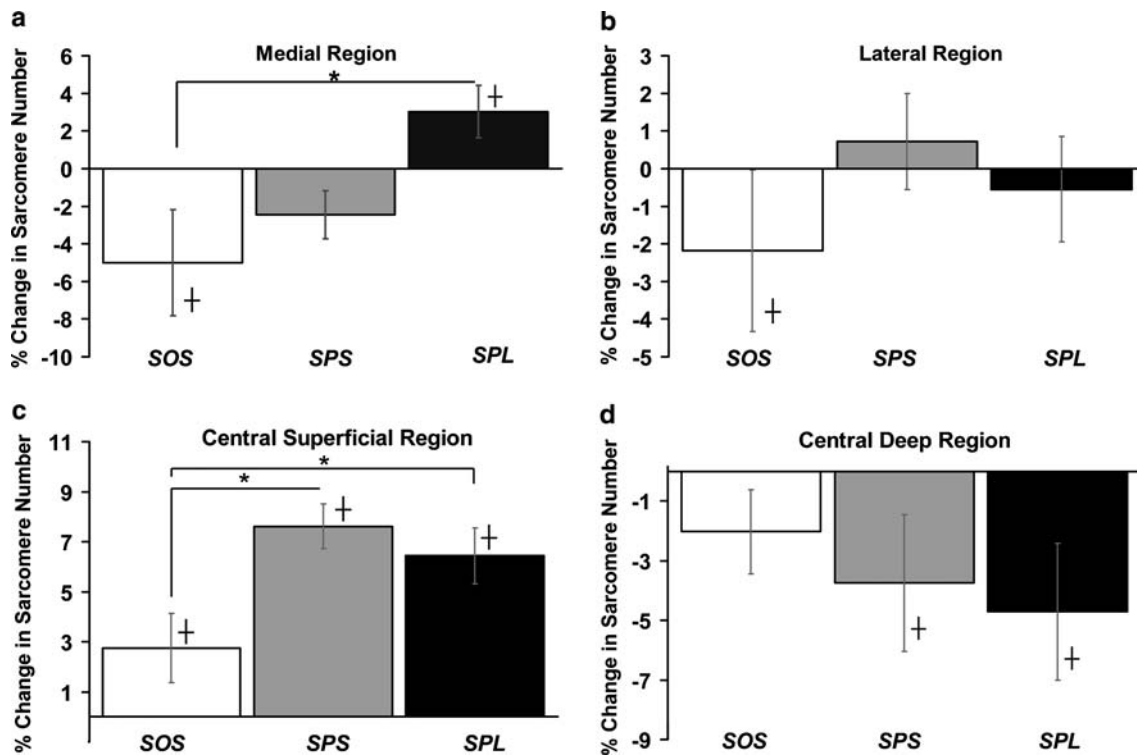


Fig. 1 Mean percent change in serial sarcomere number for four regions of the exercised tibialis anterior muscle compared to contralateral control limbs for three different exercise protocols. *open square* = SOS_c (stimulation at onset of plantar flexion at short muscle lengths); *shaded square* = SPS_c (stimulation preceding plantar flexion at short muscle lengths); and *filled square* = SPL_c (stimulation preceding plantar flexion at long muscle lengths). The *symbol* (+) indicates that the serial sarcomere number is significantly different compared to the serial sarcomere number in

the contralateral control limb. Significant differences between protocols are indicated by an *asterisk* (*). **a** Medial region; **b** Lateral region; **c** Central-superficial region; **d** Central-deep region. Note the differential adaptations in serial sarcomere numbers within the TA in all three protocols. Also, note that the medial fibers of the TA are the only fibers to adapt in a systematic fashion with respect to increasing injury in the three protocols. Values are mean \pm SE

Table 3 Alterations in serial sarcomere numbers of the central-superficial region of the tibialis anterior following chronic eccentric exercise in all rabbits, for all three chronic exercise groups

SOS _c		SPS _c		SPL _c	
Subject	% Change	Subject	% Change	Subject	% Change
rabc1	7.89	rabc10	5.58	rabc20	6.44
rabc3	0.66	rabc11	6.32	rabc21	7.38
rabc4	-3.07	rabc12	7.36	rabc22	3.05
rabc5	1.43	rabc13	7.46	rabc23	7.27
rabc7	5.53	rabc15	13.47	rabc24	10.66
rabc8	5.2	rabc16	7.14	rabc25	3.87
rabc9	1.61	rabc18	5.52		
		rabc19	8.07		
Mean ± SE	+2.7±1.4%		+7.6±0.9%		+6.4±1.1%

SOS_c stimulation at the onset of plantar flexion at short muscle lengths, SPS_c stimulation preceding plantar flexion at short muscle lengths, SPL_c stimulation preceding plantar flexion at long muscle lengths. The percentage change is calculated by dividing the serial sarcomere number change in the left (exercised) limb by the number measured in the right (control) limb

nificantly different between the three protocols ($p=0.021$). Following chronic exercise at short lengths without pre-activation (SOS_c group), peak torque was shifted $+2.3\pm 1.7^\circ$ to the right (Fig. 2), which was a significantly smaller shift compared to those measured in the SPS_c (Fig. 3) and SPL_c (Fig. 4) groups ($+7.8\pm 0.8$ and $+9.6\pm 1.9^\circ$, respectively).

Isometric torque production was increased at all joint angles following exercise in all three groups, but varied with each protocol (Fig. 5). Following eccentric exercise at short lengths without pre-activation (SOS_c group), the subsequent increase in peak isometric joint torque was similar for all the joint angles tested (Fig. 5). Chronic exercise in the SOS_c group resulted in significantly greater isometric torque improvements at short muscle lengths (55–95°) compared to the two pre-activation groups (SPS_c and SPL_c) ($p<0.05$). However, pre-activation prior to stretch (SPS_c and SPL_c) resulted in similar isometric torque gains regardless of the

starting length of the muscle, with the greatest gains at long muscle lengths. However, at joint angles of 140–150° the improvement in peak isometric torque production following exercise at long muscle length (SPL_c) was significantly greater when compared to the SPS_c group ($p<0.05$, Fig. 5).

Strain and torque as predictors of adaptation

To elucidate the possible role of individual mechanical variables on the long-term serial sarcomere number adaptation in muscle, adaptations in serial sarcomere number in the chronic exercise groups were compared directly to the mechanical variables measured in the corresponding acute protocols. Because fiber strains were only measured in the central-superficial region of the TA, only central-superficial data for sarcomere adaptation were used in the regression analyses. Individual linear regressions were performed using strain,

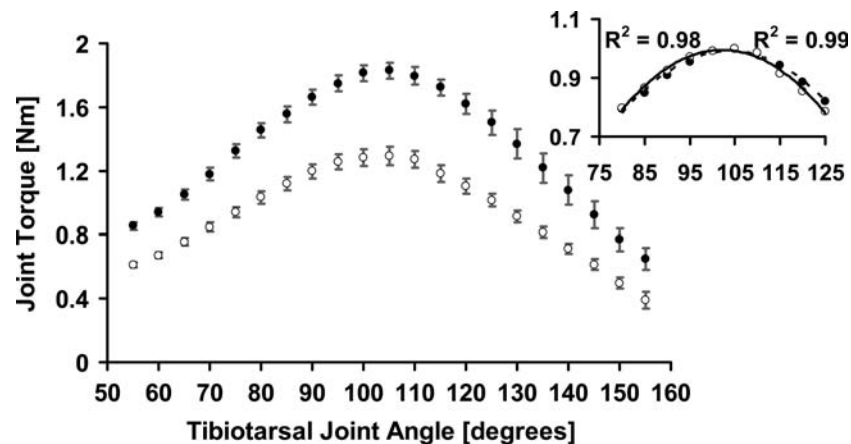


Fig. 2 Pre-exercise (open circle) and post-exercise (filled circle) torque–angle relationship for the SOS_c group. Note the shift of peak joint torque production following chronic eccentric exercise and the similarity in the shapes of the relationships, indicative of an increase in torque production at all joint angles (Fig. 5). Inset

panel: Normalized torque values greater than 75% peak torque for pre-exercise (open circle) and post-exercise (filled circle) values, fitted with a second-order polynomial, depicting the rightward shift in peak joint moment production following exercise. Values are mean ± SE

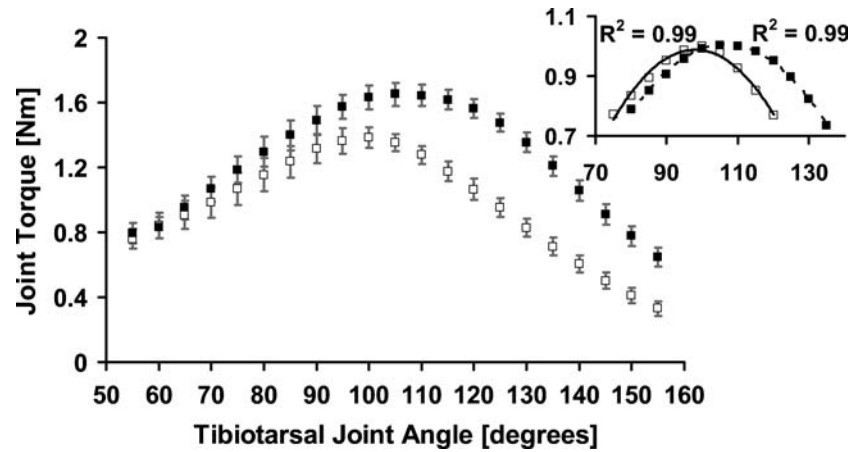


Fig. 3 Pre-exercise (*open square*) and post-exercise (*filled square*) torque–angle relationship for the SPS_c group. Note the shift of peak joint torque production and the altered shape of the relationship following chronic eccentric exercise, indicative of an increase in torque production at long muscle lengths (Fig. 5). *Inset*

panel: normalized torque values greater than 75% peak torque for pre-exercise (*open square*) and post-exercise (*filled square*) values, fitted with a second-order polynomial, depicting the rightward shift in peak joint moment production following exercise. Values are mean \pm SE

torque, and injury data from the acute groups (SOS , SPS , and SPL) as depicted in Table 1. These data were used to predict the serial sarcomere adaptations observed in the chronic exercise protocols (SOS_c , SPS_c , or SPL_c) as depicted in Table 3.

Although none of the mechanical variables that were analyzed revealed a significant relationship with the serial sarcomere number adaptations, the peak eccentric torque produced during stretch was the closest ($p=0.08$, Fig. 6e). Subsequently, the effects of numerous fiber strains on adaptation were also examined, with relaxation strain ($r^2=0.94$) and maximum fiber strain ($r^2=0.70$) being the best predictors of the serial sarcomere number adaptation (Fig. 6c, d), although neither was significant ($p < 0.05$). All other fiber strains, as well

as isometric torque reduction following exercise as a measure of injury, were poor explanatory variables for adaptation (Fig. 6, panels A, B, and F).

Discussion

The working hypotheses for this study were: (1) MTU strain is the primary factor to produce sarcomerogenesis, and therefore serial sarcomere adaptations are identical following three eccentric exercise protocols with identical MTU strain magnitude; (2) that serial sarcomere number adaptations are uniform across all regions of the muscle. To test these hypotheses, we quantified the adaptations that occur following long-

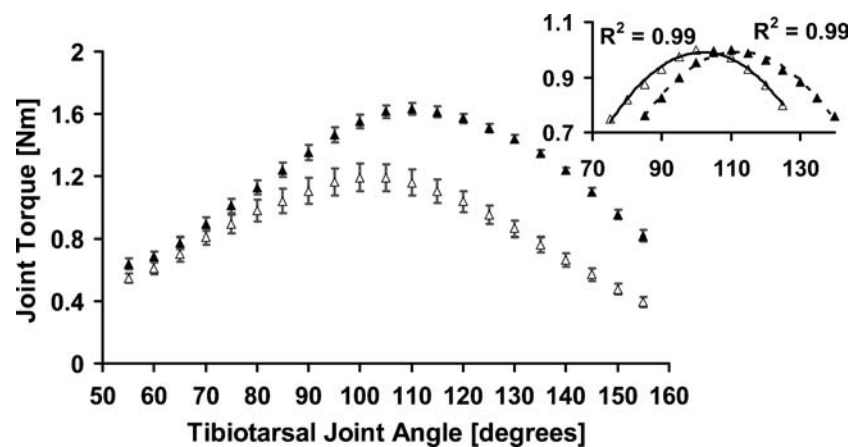


Fig. 4 Pre-exercise (*open triangle*) and post-exercise (*filled triangle*) torque–angle relationship for the SPL_c group. Note the shift of peak joint torque production and the altered shape of the relationship following chronic eccentric exercise, indicative of an increase in torque production at long muscle lengths (Fig. 5). *Inset*

panel: normalized torque values greater than 75% peak torque for pre-exercise (*open triangle*) and post-exercise (*filled triangle*) values, fitted with a second-order polynomial, depicting the rightward shift in peak joint moment production following exercise. Values are mean \pm SE

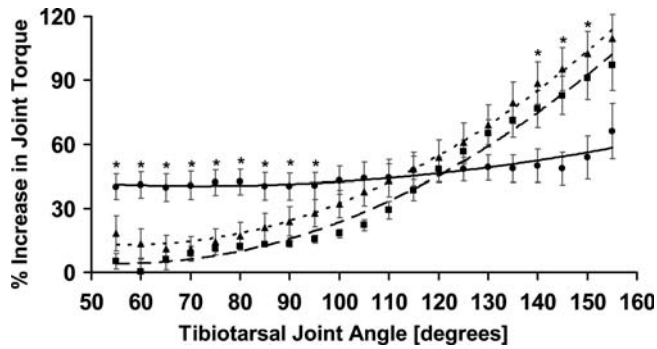


Fig. 5 Percentage increase in isometric torque for 21 tibiotarsal joint angles following 6 weeks of eccentric exercise for the SOS_c (filled circle), SPS_c (filled square), and SPL_c (filled triangle) groups. Note that following chronic eccentric exercise at short lengths without pre-activation (SOS_c), torque increase is not length specific. Pre-activation (SPS_c and SPL_c groups) results in greater torque production at long muscle lengths

term, chronic eccentric exercise, and related these adaptations to previous findings of fiber strains and joint torque produced in one acute exercise bout identical to the exercise bouts used for the chronic testing. We assume that the fiber dynamics measured in our acute protocols were about the same as those experienced by the animals of the corresponding chronic protocols. This is a tenable assumption for the first chronic exercise bout, as it was identical to the acute bout. However, following the first bout, adaptations occurring as a consequence of the chronic eccentric exercise may have altered the fiber dynamics in an unknown manner.

Differential adaptations within the tibialis anterior

Traditionally, serial sarcomere number adaptations have been studied by immobilizing the muscle in a stretched

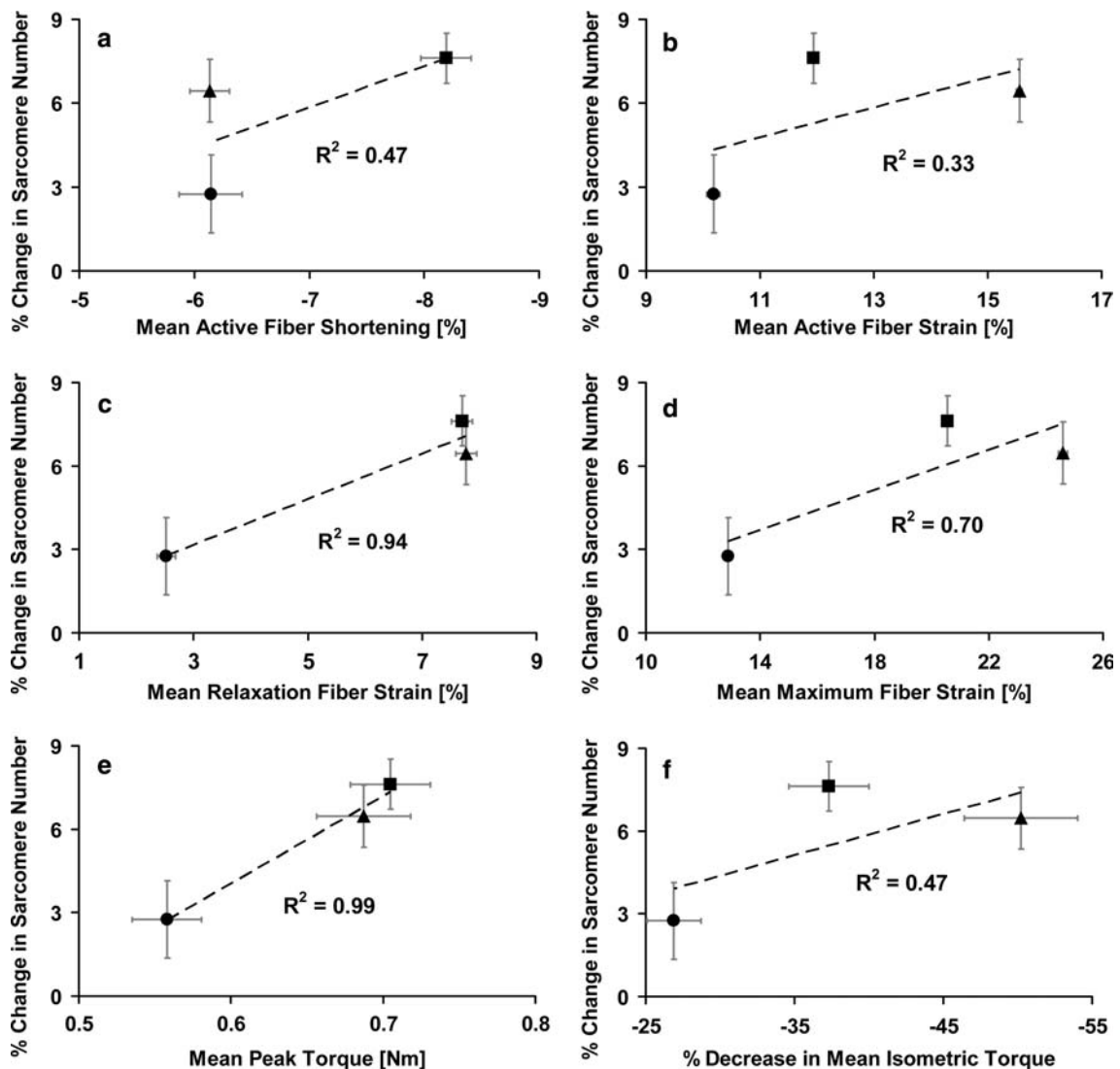


Fig. 6 Multiple, simple linear regressions to assess the predictive value of specific fiber strains and eccentric torque production on serial sarcomere number adaptation following chronic eccentric exercise in the SOS_c group (filled circle); the SPS_c group (filled

square); and the SPL_c group (filled triangle). Note that the peak torque produced (E) and relaxation fiber strain (C) during eccentric exercise best predict the increase in serial sarcomere number adaptations between the three protocols. Values are mean \pm SE

or shortened position [11]. However, the role of eccentric exercise in sarcomerogenesis has not been clearly elucidated, due, in part, to a dearth of experimental evidence. Although serial sarcomere number addition has been reported previously in the vastus intermedius (VI) of downhill walking rats [30, 31], only recently has this adaptation been associated with directly measured eccentric contractions [9]. In addition, although we observed a similar increase in serial sarcomere number in the VI muscle following chronic eccentric exercise as reported by Lynn et al. [31], the vastus lateralis (VL) did not adapt, resulting in a differential adaptation between the two synergistic muscles [9].

These differential adaptations in the rat VL and VI may point to the mechanism that produces sarcomerogenesis. Because the VI has been shown to exhibit more damage when compared to the other quadriceps muscles following downhill walking [45], the overstretching of sarcomeres and the subsequent fiber necrosis have been proposed as a possible stimulus for serial sarcomere number adaptation [38, 42]. As a predominantly slow twitch muscle, the VI may be preferentially activated during downhill walking, thus enduring greater stress and injury than the other quadriceps muscles [45]. In addition, the VI has shorter muscle fibers than the VL [9], and may experience more strain and injury during a given amount of MTU stretch when compared to the long fibers of the VL [3, 26]. Therefore, we propose that eccentric contractions may cause sarcomerogenesis in a dose dependent manner; that is, the greater the stress and strain (probably up to some limiting value), the greater the sarcomerogenesis.

Differential sarcomere number adaptations have also been observed previously within a given muscle [19]. Because the authors controlled the excursion and activation during eccentric exercise, it seems reasonable that the stress was constant throughout the muscle, although fiber dynamics would be expected to vary [10]. Similar to the argument made above, it is possible that the architectural differences between various regions of the same muscle may influence fiber strains, resulting in a differential adaptive response, as the fiber and sarcomere lengths were nonuniform throughout the four regions that were measured prior to exercise (in the contralateral control muscles). This result illustrates that, for a given MTU length change, sarcomeres in different regions of a muscle may work over different sarcomere lengths and regions of their individual force-length relationships, experiencing different strains as they are stretched during eccentric contraction.

Differential adaptations between protocols

In each of the protocols used in this study, the mechanical stimulus applied to the MTU was the same, and corresponded to a muscle strain of 5%. However, serial sarcomere number adaptation was different in all four regions for the three protocols. Specifically, in the

central-superficial region, serial sarcomere numbers increased in every protocol, while in the central-deep region, serial sarcomere numbers decreased. In the lateral region, serial sarcomere numbers decreased or did not change significantly. In the medial region, serial sarcomere numbers increased in one protocol, while they decreased for another protocol.

Previously, the stimuli for serial sarcomere number adaptations had been associated with whole muscle dynamics, even though it had been proposed that the control for sarcomerogenesis resides in the fibers [50]. It is possible that the same regions of the tibialis anterior experienced differential strains between protocols, causing different magnitudes of injury. Unfortunately, our measures of injury were for the muscle as a whole, not for the separate regions. However, if serial sarcomere number addition is a result of muscle injury, then we would propose that the central-superficial region, which gained serial sarcomeres in every protocol, would be the most susceptible to injury. Conversely, the central-deep and lateral regions would appear to be the most shielded from strain and injury, as these regions either lost serial sarcomeres or experienced no significant changes. The adaptations in the medial aspect of the tibialis anterior are more difficult to interpret, as the serial sarcomere loss resulting from the first protocol would indicate that the protocol produced no injury in that region, while the exercise at long muscle lengths produced injury, as the medial aspect gained serial sarcomeres. This result is possible, as whole muscle and isolated fibers have been shown to be more susceptible to injury when stretched at long compared to short lengths [17, 53].

Predictors of sarcomere number adaptation

Muscle injury was significantly increased in conjunction with an added pre-activation as well as an increase in starting muscle length prior to exercise. In addition, several measures of fiber strain [8] also increased accordingly, but these were only measured in the central-superficial region of the muscle. Therefore, one limitation of this study is that we can only directly relate central-superficial adaptations to the measured mechanical variables. The two best predictors of serial sarcomere number changes in our study were peak eccentric torque and relaxation strain produced during the eccentric exercise protocols. Interestingly, relaxation strain occurs during MTU shortening, and is produced by series elastic elements that store elastic energy during the active lengthening as force increases in the muscle, then release it upon deactivation (and the associated decrease in force) and muscle shortening [10]. Recently, it has been proposed that longitudinal growth of muscle depends on passive strain [51] or passive stress [60]. We can reasonably assume that the stress was uniform throughout the muscle, as we controlled and supra-maximally stimulated the tibialis anterior during all

exercise bouts. It is possible that peak eccentric torque production could serve to maximize the energy stored in series elastic elements, potentially increasing relaxation strain [10] and influencing serial sarcomere number.

Although differential muscle injury may influence serial sarcomere number adaptation, we found a poor relationship between the two variables, as adaptations in the central-superficial region did not correspond to the differences in injury between the three protocols. However, this is a difficult relationship to formulate, essentially comparing a global measure of muscle injury to an associated regional adaptation. Similarly, although peak eccentric torque was an excellent predictor of adaptation, this too was a comparison of a global variable (torque) to a regional adaptation (sarcomere number adaptation). Even though eccentric exercise has long been associated with muscle injury and the delayed onset of muscle soreness, a muscle fiber can adapt in the absence of severe injury and contractile element damage [4, 12, 18, 23]. The control of serial sarcomere number at the cellular level allows fibers to respond independently, and could explain the differential adaptations within the muscle as well as between the groups observed in this study.

Possible mechanisms of serial sarcomere number adaptation

Although we only measured fiber dynamics in the central-superficial region of the tibialis anterior, we would propose that the lateral and central-deep regions of the muscle probably experienced smaller fiber strains compared to the central-superficial region. Although this may be attributed to architectural differences in the fiber and sarcomere lengths between the four regions of the TA, the relationship would be counterintuitive, as the lateral region of the tibialis anterior has longer fiber and sarcomere lengths at rest when compared to the central-deep region. Although the lateral region does not adapt as expected, the central-deep region actually lost sarcomeres.

Alternatively, the concentration of extracellular components of skeletal muscle, such as intermediate filaments [40, 47] and connective tissue [24], may reduce the susceptibility of the muscle to strain induced injury. It has been shown that fibers that are shielded from stress not only lose serial sarcomeres, but also simultaneously increase the proportion of connective tissue [55, 57]. Muscle with high concentrations of desmin are more resistant to eccentric exercise induced injury [41], which may explain why the cytoskeleton is rapidly remodeled following eccentric exercise [1, 2]. It is not known if the structural components of the extracellular matrix vary between regions of the tibialis anterior, however, this may help to explain the differential adaptations we found in this study. Increased stiffness of the fibers would be expected to reduce the relaxation strains and would make increased serial sarcomere number additions unnecessary.

Summary

This was the first study to show the effects of chronic eccentric exercise on serial sarcomere number in four separate regions of the tibialis anterior. We observed differential adaptations in four distinct regions of the tibialis anterior muscle in response to chronic eccentric exercise. By adjusting the starting muscle length and activation timing prior to stretch, we demonstrated that serial sarcomere number adaptations can be significantly altered without changing the mechanical strain to the MTU, illustrating that fiber strains are a more powerful stimulus for adaptation than MTU stress and strain. The differential adaptation in the muscle may be explained by the different architecture of the individual fibers in different regions of the muscle, causing different strains in fibers from different regions for a given change in tibiotarsal joint angle.

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