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Effect of altering starting length and activation timing of muscle on fiber strain and muscle damage

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Butterfield, Timothy A., and Walter Herzog. Effect of altering starting length and activation timing of muscle on fiber strain and muscle damage. *J Appl Physiol* 100: 1489–1498, 2006. First published January 5, 2006; doi:10.1152/jappphysiol.00524.2005.—Muscle strain injuries are some of the most frequent injuries in sports and command a great deal of attention in an effort to understand their etiology. These injuries may be the culmination of a series of subcellular events accumulated through repetitive lengthening (eccentric) contractions during exercise, and they may be influenced by a variety of variables including fiber strain magnitude, peak joint torque, and starting muscle length. To assess the influence of these variables on muscle injury magnitude *in vivo*, we measured fiber dynamics and joint torque production during repeated stretch-shortening cycles in the rabbit tibialis anterior muscle, at short and long muscle lengths, while varying the timing of activation before muscle stretch. We found that a muscle subjected to repeated stretch-shortening cycles of constant muscle-tendon unit excursion exhibits significantly different joint torque and fiber strains when the timing of activation or starting muscle length is changed. In particular, measures of fiber strain and muscle injury were significantly increased by altering activation timing and increasing the starting length of the muscle. However, we observed differential effects on peak joint torque during the cyclic stretch-shortening exercise, as increasing the starting length of the muscle did not increase torque production. We conclude that altering activation timing and muscle length before stretch may influence muscle injury by significantly increasing fiber strain magnitude and that fiber dynamics is a more important variable than muscle-tendon unit dynamics and torque production in influencing the magnitude of muscle injury.

skeletal muscle; muscle mechanics; lengthening contractions

MUSCLE STRAIN INJURIES ARE the most frequent injuries in sports (20). As a result, they have commanded a great deal of attention in an effort to understand their etiology. Over the years, several risk factors have been proposed, including muscle weakness and fatigue (20, 21), malfunctioning neuromuscular coordination pattern (1, 21), inadequate flexibility (29, 53), improper warm-up (10, 48), and inadequate strength or endurance (15, 29), among others. However, the relationship between these proposed strain injury etiologies and the muscle injury mechanisms gleaned from *in situ* and *in vitro* experiments in the past has not been firmly established.

Historically, experimental protocols used to elucidate the mechanisms of severe disruptive muscle injury have focused on high magnitudes of force and strain. To assess the function of muscle after severe disruptive muscle strain injuries, isolated muscles (37, 50, 54) or fibers (4, 5, 59, 60) are preactivated and subjected to a minimal number of stretches beyond physiological range to produce a severe, disruptive strain

injury. These protocols have been instrumental in elucidating relationships between mechanical factors, muscle strain injury, and the subsequent physiological response. Although such studies have been essential to our understanding of severe muscle strain injuries, the fact that these are produced utilizing a nonphysiological range of motion makes application to *in vivo* muscle function difficult.

Conversely, strain-induced subcellular damage results in delayed onset muscle soreness, does not result in immediate pain and disability, and appears to limit the subsequent fibrosis, allowing regeneration and repair that may improve the function of the muscle. Most *in vivo* muscle injury protocols have resulted in nondisruptive, subcellular damage after eccentric exercise in either human (38–40) or animal models (3, 16, 28, 32). When using *in vivo* protocols within a physiological range of motion, more than one repetition is required to produce muscle injury (16, 23, 57). This may be due to the disassociation between fiber dynamics and muscle-tendon unit (MTU) dynamics that have been observed during locomotion (24, 26). Muscles acting as compliant actuators (61) tend to store and release energy during locomotion, which serves to increase the efficiency of the musculoskeletal system (25). This type of behavior is not represented well during *in vitro* and *in situ* eccentric contraction protocols and may be a key feature in the difference between disruptive strain injury and subcellular damage *in vivo*. Recently, it has been proposed that the accumulation of subcellular damage through repetitive fiber strain may lead to progressive MTU injury, culminating in an acute, observable muscle strain injury in athletes (9).

It is well accepted that muscle strain injuries occur while the muscle is activated and lengthening (13, 17–19, 21, 31, 34, 36, 62). Utilizing lengthening contractions (eccentric contractions), animal models have provided great insight into the cellular mechanisms of muscle strain injury. Traditionally, two unique mechanical properties of lengthening contractions have been associated with strain induced muscle injury: high peak force production (30, 37, 54) and fiber strain magnitude (34, 35). With the use of these ideas, subcellular damage has been proposed to result from a sequence of events that may begin with either cytoskeletal disruption (6, 34) or unstable sarcomeres (42, 51). The loss of structural integrity of the fibers, in combination with subsequent active lengthening contractions, may result in increasing injury with time (22). In this fashion, microscopic subcellular damage may ultimately present clinically as muscle pain and weakness, as fiber necrosis progresses to inflammation and impairment (8, 9, 45).

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Starting muscle length before stretch has been shown to influence muscle injury, with a longer starting length resulting in a greater magnitude of muscle injury in *in vitro* (23, 52, 58), *in situ* (55), and *in vivo* (16, 39) protocols. This may be due to several factors: first, stretch at longer muscle lengths may produce greater peak force compared with stretch at shorter muscle lengths, and this has been associated with increases in strain injury (37); and, second, at very long muscle lengths the overextension of sarcomeres within fibers may result in contractile element injury, propagating to fiber necrosis and weakness (9, 45, 58), indirectly implicating fiber length in muscle strain injury.

The association between activation timing and muscle strain injury has also been proposed as an etiology for muscle strain injury (7), but the exact mechanisms are unknown. Although there is a dearth of evidence supporting this hypothesis, an *in situ* approach by Stevens (50) showed that altering the timing of activation during stretch-shortening cycles influenced the magnitude of muscle injury in rat hindlimb muscles. In addition, activation timing has been shown to influence peak force production during lengthening contractions (34), which potentially provides a link between activation timing and muscle fiber injury due to high peak force production during lengthening contractions.

Ultimately, the effects of activation timing and starting muscle length on peak force and fiber dynamics during stretch-shortening cycles remain unknown. Therefore, the purpose of this paper was threefold: first, to determine the effects of small changes in the timing of activation on fiber dynamics and joint torque production during *in vivo* stretch-shortening cycles at short muscle lengths; second, to compare the fiber dynamics and joint torque during stretch-shortening cycles at short muscle length with those measured during an identical stretch-shortening protocol at long muscle length; and lastly, to elucidate the potential relationship between peak force, fiber strain, and muscle injury.

METHODS

Surgical procedure. Muscle and fiber strain was determined from both hindlimbs of 18 skeletally mature female New Zealand White rabbits (5.5 ± 0.2 kg, Riemens, St. Agatha, ON, Canada). Rabbits were obtained and studied with the approval of the Animal Care Committee of the University of Calgary and were housed locally in accordance with Canadian Council on Animal Care Guidelines. Rabbits were tranquilized with 0.18 ml (10 mg/ml) acepro-25 (MTC Pharmaceuticals, Cambridge, ON, Canada) and held under anesthesia with 1.5% isoflurane, 0.6 l/min N_2O , and 0.8 l/min O_2 . Incisions were made on the posterior aspect of the hindlimbs, anterior to the sciatic vein, and the biceps femoris and semimembranosus were separated exposing the peroneal nerve. Nerve cuff stimulating electrodes were secured over the right and left common peroneal nerves, superior to the gastrocnemius and distal to the branching of the sciatic nerve. In this manner, all dorsiflexors of the tibiotarsal joints were stimulated effectively.

To measure fascicle lengths, an incision was made over the anterior aspect of the shank, exposing the tibialis anterior and extensor digitorum longus. The fascia was removed from the anterior aspect of the tibialis anterior (TA), and a superficial fascicle was isolated by microstimulation (12). Piezoelectric crystals (Sonometric, London, ON, Canada), 2 mm in diameter, were sutured to the proximal and distal ends of the fascicle in the central, superficial region. After implantation, the skin was stapled closed.

Fiber strain measurements. Rabbits were divided into three groups, based on starting muscle length and 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 longer muscle length (95° tibiotarsal joint angle). Lengthening contractions in all three groups were performed through a range of motion that resulted in a $\sim 5\%$ strain to the MTU, or 35° range of motion in the SOS and SPS groups. Because the TA moment arm decreases as the tibiotarsal joint is plantar flexed, the tibiotarsal joint angle change needed to produce a $\sim 5\%$ MTU strain in the SPL group was 50° .

Setup and experimental protocols. For all three groups, rabbits were first placed supine in a stereotaxic frame with the knee joint at 90° . The left foot was strapped to a servo-motor foot plate (Parker Hannifin, Irwin, PA) that was controlled via computer (Motion Planner, Rohnert Park, CA). The tibiotarsal angle was set at 90° (increased tibiotarsal joint angle = increased plantar flexion), which served as the reference angle for the remainder of the experiment. The peroneal nerve cuff leads were attached to a stimulator (Grass S8800, Astro-Med, Longueuil, QC, Canada), and the α -motoneuron threshold was determined (pulse duration = 0.1 ms, frequency = 150 Hz, train duration = 500 ms).

First, preexercise isometric ankle torque was measured by supra-maximally stimulating ($3 \times \alpha$ -motoneuron threshold voltage, pulse duration = 0.1 ms, frequency = 150 Hz, train duration = 2,000 ms) the dorsiflexor muscles, beginning at a tibiotarsal angle of 55° and progressing in 5° increments to 155° . The foot was returned to a dorsiflexed position (55° tibiotarsal angle) for 2 min of rest between contractions. Then, cyclic lengthening contractions were performed from a tibiotarsal angle of 70 to 105° of plantar flexion at $70^\circ/s$ for the SOS and SPS groups and from 95 to 145° of plantar flexion at $100^\circ/s$ for the SPL group. Tibiotarsal torque was measured from strain gauges placed on the cam between the servo-motor and foot plate. The protocol consisted of five sets of ten cyclic lengthening contractions, with 2 min of rest between sets.

Afterward, isometric joint torque was once again measured for the 21 tibiotarsal joint angles (55 – 155° in 5° increments) for direct comparison to the preexercise values. To calculate the angle of peak joint torque for each rabbit, all values $>75\%$ of the absolute peak joint torque were normalized and fit with a second order polynomial, and peak joint torque was calculated as the peak value of the polynomial approximation (11). Individual shifts in the torque-joint angle relationships were calculated by comparing the angle of peak isometric joint torque postexercise to the angle of peak isometric joint torque production preexercise. The mean shift in peak joint torque production was calculated for each exercise group (SOS, SPS, SPL).

Data collection. All data were collected via Sonosoft data-acquisition systems (Sonometrics, London, ON, Canada) at 498 samples per second. In addition, strain gauge signals were also recorded using WinDaq data-acquisition software (Dataq instruments, Akron, OH). Torque signals were low-pass filtered at 20 Hz through a second order recursive Butterworth filter (Intertechnology, Don Mills, ON, Canada). Fiber lengths during the entire protocol were measured using sonomicrometry with the two piezoelectric crystals inserted in a central, superficial fascicle (14, 24). Because fibers of the TA do not run the entire length of the muscle (33), we measured fascicle length. Thus the term "fiber" in this paper refers to fascicles that span the entire length of the TA from the distal to proximal aponeurosis. Percent fiber strain was calculated using Eq. 1.

$$\frac{T - T_o}{T_o} \times 100 \quad (1)$$

Briefly, the piezoelectric crystals can transmit and receive sound waves, allowing a measurement of the time interval between sound wave propagation from one crystal and reception by the other crystal. Because sound travels at a constant velocity (V) in both passive and contracting mammalian skeletal muscle (14), the distance between the two crystals (L), or the fascicle length, is simply a function of time, based on Eq. 2.

$$L = V \times \Delta T \quad (2)$$

Thus the difference (ΔT) between the instantaneous ultrasound transmission time (T) and the transmission time at the muscle fiber reference length just before starting the MTU activation and stretch (T_o) provides the change in fiber length during contraction. MTU length change was determined by using a tendon travel approach (2) after animals were killed.

Because of the complex fiber dynamics during eccentric contractions, we defined the stretch and shortening cycles of the fibers in all three groups with respect to Fig. 1: active shortening, period of fiber shortening at the onset of MTU stretch as force is increasing ($B_r - A_r$); active strain, period of fiber stretch during the eccentric MTU phase ($C_r - B_r$); relaxation strain, period of fiber stretch at the beginning of passive MTU shortening, when muscle force is decreasing ($D_r - C_r$); passive shortening, period of fiber shortening during passive MTU shortening ($A_{r+1} - D_r$). Lastly, maximum strain was calculated as the shortest fiber length subtracted from the longest fiber length during each repetition (Fig. 1, $D_r - B_r$) and included both active and relaxation strains. Because of the change in activation timing for the SPS and SPL groups, active shortening of the fibers occurred during an isometric contraction of the MTU and preceded plantar flexion of the tibiotarsal joint by 100 ms. In addition, we kept activation duration constant at 500 ms between all three groups to minimize the confounding effects of fatigue between protocols. Thus

the alteration of timing activation used in this study is associated with a complete shift of activation duration with respect to joint motion. Therefore, although active shortening occurs as a 100-ms isometric contraction in the SPS and SPL groups, the relaxation strain occurs during 100 ms of passive plantar flexion in these groups, whereas relaxation strain occurs during passive dorsiflexion in the SOS group.

Statistics. All statistical analyses were performed using SPSS version 12.0 (SPSS, Chicago, IL). For all parameters measured, means and SE are reported. A two-way analysis of variance was used to assess the effects of both activation timing and set on fiber strain magnitude. Significant interaction effects were analyzed by using a multivariate analysis of variance with a least squared differences post hoc analysis. To measure the difference in torque production and torque decrement between groups, a Kruskal-Wallis χ^2 test was used. After the exercise protocol, MTU injury was measured using three different techniques: 1) by quantifying the rightward shift in the ankle torque joint-angle relationship, 2) by measuring the isometric joint torque decrement at 21 distinct tibiotarsal joint angles, and 3) by measuring the reduction in isometric joint torque production at optimal muscle length. Statistical significance was set at $P < 0.05$.

RESULTS

During the stretch-shortening cycles the mechanical strains experienced by the MTU were similar between all three groups ($P = 0.973$). Mean MTU strains were $5.0 \pm 0.2\%$ for the SOS group, $5.0 \pm 0.5\%$ for the SPS group, and $5.2 \pm 0.7\%$ for the SPL group. Although activation timing and muscle length were altered, fiber dynamics were qualitatively similar between all three protocols, as fibers from all groups underwent a four-phase length change during one complete stretch-shortening cycle (Fig. 1), regardless of activation timing and starting muscle length. In addition, we observed that active fiber shortening diminished with each repetition in each set and



Fig. 1. *Top*: representative raw data trace of *repetition 1* showing fiber strain during one MTU stretch-shorten cycle for the SOS group (solid lines), SPS group (dashed line), and SPL group (dotted line). Subscript *r* represents eccentric-concentric repetition number. A_r , starting length of the fibers before each lengthening contraction. B_r , shortest length of the fiber after the active shortening (A_r to B_r) during the eccentric contraction. Active strain is defined as the change of fiber length during the eccentric phase from B_r to C_r ($C_r - B_r$). During the concentric, deactivated phase, the relaxation strain occurs from point C_r to point D_r , and the corresponding length change is calculated by $D_r - C_r$. After the relaxation strain, the fibers shorten as the MTU continues to shorten during the return of the foot to the initial, dorsiflexed position, and to point A_{r+1} of the next repetition, point A_{r+1} . *Down arrows* indicate start of activation. *Middle*: corresponding raw data trace of *repetition 1* showing joint-torque during 1 muscle-tendon unit (MTU) stretch-shorten cycle for the SOS group (stimulation at onset of stretch, with lengthening contractions starting at a short muscle length; solid lines), SPS group (stimulation preceding stretch by 100 ms, with lengthening contractions starting at a short muscle length; dashed line), and SPL group (stimulation preceding stretch by 100 ms, with lengthening contractions starting at a longer muscle length; dotted line). *Bottom*: illustration of MTU length change for SOS group (solid line) and SPS and SPL groups (dashed line).

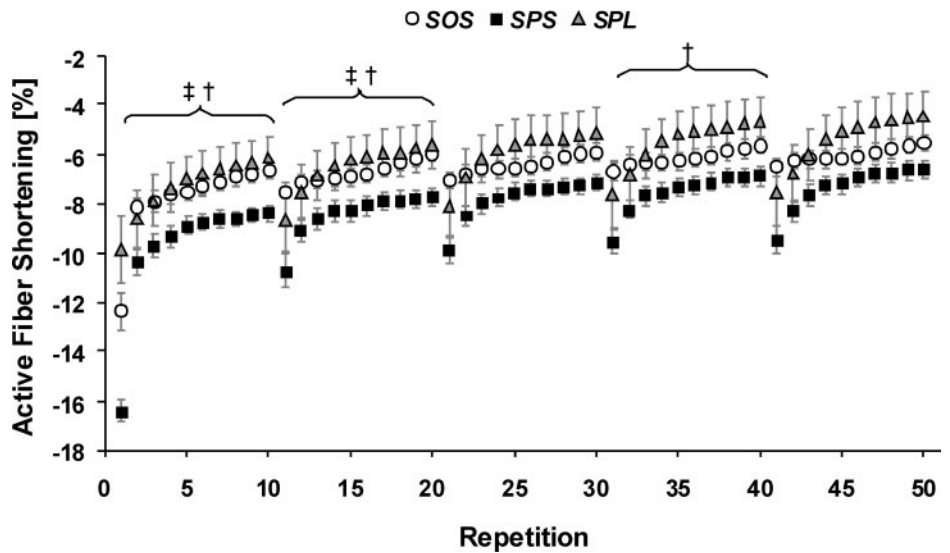


Fig. 2. Mean active fiber shortening (defined as $B_r - A_r$, Fig. 1) for all 50 repetitions in the SOS group (○), the SPS group (■), and the SPL groups (▲). Overall, altering the starting length of the muscle before stretch after preactivation had no effect on active fiber shortening, because the SPS and SPL groups were not significantly different. However, preactivation at short muscle length resulted in greater active shortening of the fibers compared with the SOS group in sets 1 and 2 (‡) and compared with the SPL group in sets 1, 2, and 4 (†).

recovered at the start of the following set. Comparison of joint torque and fiber length changes in these groups (data not shown) was consistent with our previous findings of an increasing compliance within the contractile element during repeated stretch-shortening cycles between sets for all groups (12).

Initially, fibers shortened upon activation and force production for all groups. Active fiber shortening occurred while the MTU was lengthening in the SOS group but occurred while the MTU length was constant in the SPS and SPL groups (Fig. 1, $B_r - A_r$). Mean active fiber shortening was not significantly different between the three exercise groups ($P = 0.081$); however, active fiber shortening did vary significantly within groups, among sets ($P < 0.001$). Preactivation at long muscle lengths (SPL) resulted in significantly less active shortening compared with preactivation at short muscle lengths (SPS) in sets 1, 2, and 3 ($P < 0.05$, Fig. 2). A significant interaction of activation timing and set ($P = 0.038$) indicated that preactivation before stretch (SPS and SPL groups) resulted in a greater reduction in active shortening over time compared with the SOS group (Fig. 2).

Active fiber strain (Fig. 1, $C_r - B_r$) was calculated during activation and lengthening of the MTU (lengthening contrac-

tion). Therefore, active strain in the SOS group ceased at the end of plantar flexion, or at a tibiotarsal joint angle of 105° (Fig. 1). Because of the offset of activation in the SPS and SPL groups, active strain ceased 100 ms before the end of plantar flexion. This occurred at a tibiotarsal joint angle of 98° for the SPS group and at a tibiotarsal joint angle of 135° for the SPL group. Simply altering the timing of activation at short muscle lengths did not significantly increase active fiber strain between the SOS and SPS groups ($P = 0.065$), but increasing starting length increased active fiber strain (SPL) compared with both SOS ($P < 0.001$) and SPS ($P = 0.002$) groups. Within groups, active fiber strains differed among sets ($P < 0.001$); however, there were no interaction effects between group and set ($P = 0.625$, Fig. 3).

Relaxation strain occurred at the cessation of activation in all three protocols. In the SOS group, relaxation strain occurred while the MTU was shortening, but in the SPS and SPL groups relaxation strain occurred while the MTU was lengthening for an additional 100 ms ($D_r - C_r$, Fig. 1). Relaxation strains differed significantly between the groups ($P = 0.001$) as well as within groups ($P < 0.001$), although the relaxation strains of both preactivation groups (SPS and SPL) were nearly identical (Fig.

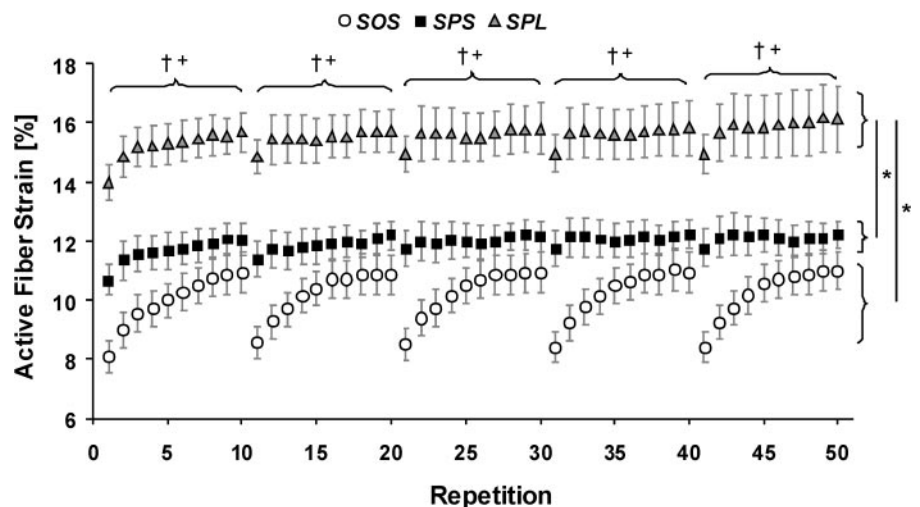


Fig. 3. Mean active fiber strain (defined as $C_r - B_r$, Fig. 1) for all 50 repetitions in the SOS (○), SPS (■), and SPL (▲) groups. Between groups, preactivation of the muscle before stretch at long muscle lengths resulted in significantly greater active fiber strains for all 50 repetitions compared with both exercise groups beginning at short muscle lengths. However, preactivation at short lengths did not significantly alter mean active strain values. Within sets, all active strain values obtained by exercise at long lengths (SPL group) were greater than those of the SOS (+) and SPS (†) groups. *Differences between groups.

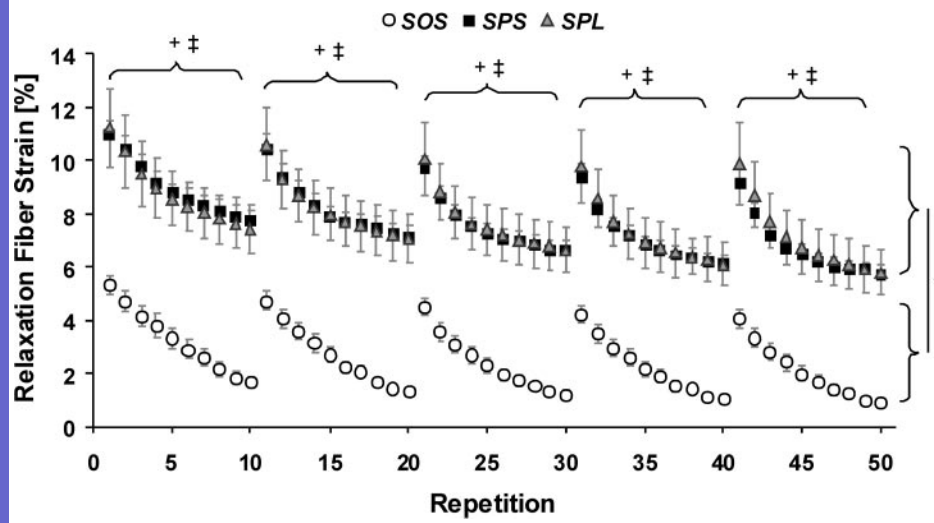


Fig. 4. Mean relaxation fiber strain (defined as $D_r - C_r$, Fig. 1) for all 50 repetitions in the SOS (\circ), SPS (\blacksquare), and SPL (\blacktriangle) groups. Between groups, preactivation of the muscle before stretch (SPS and SPL groups) resulted in significantly greater relaxation fiber strains for all 50 repetitions compared with exercise beginning at short muscle without preactivation (SOS group). Within sets, preactivating the muscle before stretch (SPS, SPL) resulted in significantly greater relaxation fiber strains compared with the SOS group (+, ‡) for every set. However, there was no significant difference in relaxation fiber strains in the preactivation groups in any set, regardless of starting muscle length (SPL compared with SPS). *Differences between groups.

4). Thus mean relaxation strains in both preactivation groups (SPS and SPL) were significantly greater than the SOS group ($P < 0.001$) but not significantly different from each other ($P = 0.884$, Fig. 4). The significant interaction effect ($P < 0.001$) indicated that relaxation strains significantly diminished over time but to a greater extent in the preactivation groups.

Maximum fiber strain [the sum of active and relaxation fiber strains ($D_r - B_r$, Fig. 1)] was significantly altered by preactivation. Preactivating the muscle before stretch at short lengths (SPS) significantly increased maximum strain compared with the SOS group ($P < 0.001$), whereas increasing starting muscle length also resulted in significantly greater maximum fiber strain compared with both the SOS ($P < 0.001$) and SPS groups ($P = 0.036$). There was a significant interaction of exercise group and set ($P < 0.001$), indicating that although maximum fiber strains in the preactivation groups (SPS and SPL) differed from the maximum fiber strains in the SOS group, these differences varied between sets for the duration of the exercise (Fig. 5).

Torque joint-angle relationship. Peak isometric joint torque was reduced at all tibiotarsal joint angles in all three groups, and the shape of the torque-angle curve was altered after the

exercise protocols (Figs. 6–8). After the exercise bouts, peak joint torque was produced at a greater tibiotarsal joint angle (corresponding to a longer muscle length) within each group compared with preexercise values in the SOS group ($+7.7 \pm 1.9^\circ$, $P = 0.028$, Fig. 6), the SPS group ($+7.9 \pm 1.8^\circ$, $P = 0.01$, Fig. 7), and the SPL group ($+10.9 \pm 1.0^\circ$, $P < 0.001$, Fig. 8). However, these rightward shifts were not significantly different between the three groups ($P = 0.122$).

Isometric joint torque decrement at various tibiotarsal joint angles. After the eccentric exercise protocol, the reduction in mean isometric joint torque was not significantly different between any of the three groups at the shortest muscle length, which corresponded to a tibiotarsal joint angle of 55° ($P = 0.334$, Fig. 9). However, applying a preactivation before stretch at short muscle lengths (SPS) and long muscle lengths (SPL) significantly reduced postexercise isometric joint torque at tibiotarsal joint angles of 60° and greater compared with the SOS group. Exercise at long lengths (SPL) resulted in the greatest reduction in postexercise isometric joint torque at all tibiotarsal joint angles $>60^\circ$ (Fig. 9).

Reduction in isometric joint torque at optimal length. The tibiotarsal joint angle at which the peak joint torque was

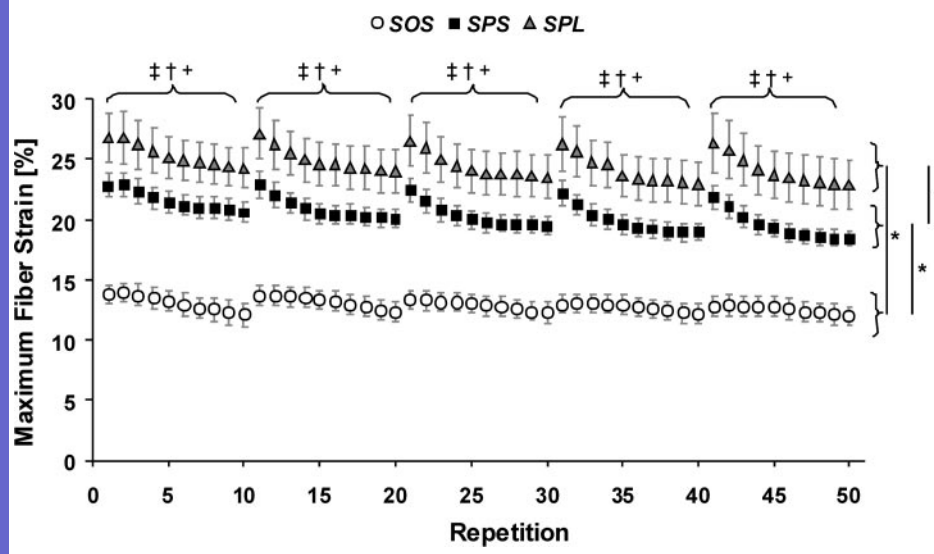


Fig. 5. Mean maximum fiber strain (defined as $D_r - B_r$, Fig. 1) for all 50 repetitions in the SOS (\circ), SPS (\blacksquare), and SPL (\blacktriangle) groups. Maximum strain is the sum of active strain and relaxation strain. Maximum strain was significantly different between all 3 groups and within all 5 sets. Preactivation before stretch at short muscle lengths significantly increased maximum strain compared with the values obtained from stretch without preactivation at the short muscle length (††). However, preactivation at the long muscle length resulted in significantly greater maximum strains compared with exercise at short length, both with (†) and without (+) preactivation. *Differences between groups.

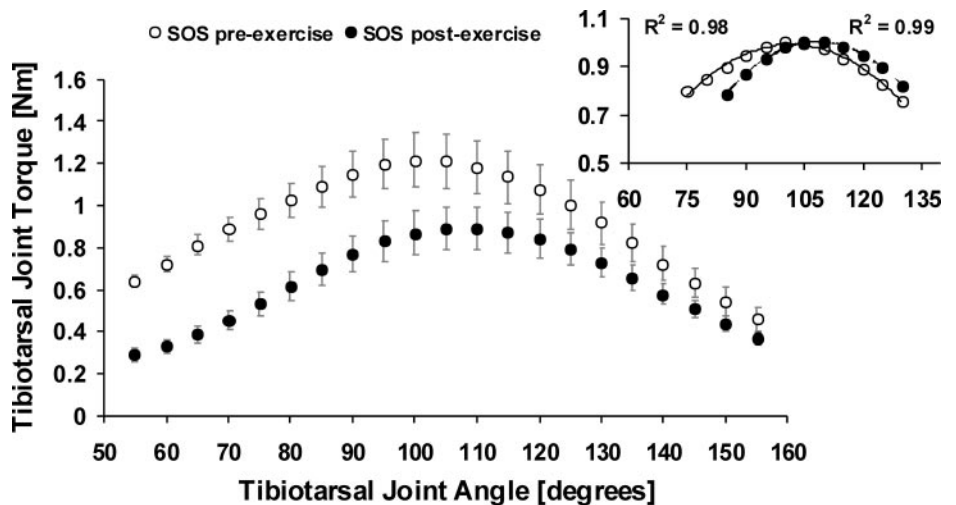


Fig. 6. Preexercise (\circ) and postexercise (\bullet) torque-joint angle relationship for the SOS group. Note the shift of peak joint torque production to a greater joint angle and a change in the shape of the relationship after the exercise bouts. *Inset*: normalized values $>75\%$ peak torque for pre- (\circ) and postexercise (\bullet) values in each group were fitted with a second order polynomial, depicting the rightward shift in peak joint torque production after exercise ($+7.7 \pm 1.9^\circ$).

produced shifted rightward after the exercise protocol in all three groups (Figs. 6–8). Therefore, we identified this new optimum muscle length after exercise and selected the corresponding peak isometric joint torque for comparison to the preexercise values. The mean reduction in peak joint torque after the lengthening contraction bouts was significantly different between groups ($P = 0.003$). The values were $26.9 \pm 1.8\%$ for the SOS group, $37.3 \pm 2.7\%$ for the SPS group, and $50.2 \pm 3.8\%$ for the SPL group. At short muscle lengths, a small alteration in the timing of muscle activation (SPS group) resulted in a significantly greater reduction in peak isometric joint torque compared with exercise at short muscle lengths without preactivation (SOS group). Additionally, increasing the starting length of the muscle before the cyclic stretch-shortening protocol also resulted in a significantly greater reduction in peak isometric joint torque production compared with both short muscle length groups, with (SPS) and without (SOS) preactivation.

Peak joint torque during stretch-shortening cycles. Peak joint torque produced during the cyclic stretch-shortening cycles was produced at the longest fiber length magnitudes during activation, corresponding to the end of active fiber strain (*point C*, Fig. 1). Preactivating the muscle before stretch at short muscle lengths (SPS group) resulted in a significant increase in

joint torque for all repetitions of the first three sets (Fig. 10, $P < 0.05$). In *sets 4* and *5*, preactivation resulted in significantly greater joint torque for only the last five and six repetitions, respectively (Fig. 10). Increasing the starting muscle length before stretch (SPL) resulted in greater joint torque production in *sets 2* and *3* compared with the SOS group ($P < 0.05$). However, comparing the preactivation groups (SPS, SPL), increasing starting length (SPL) did not result in greater joint torque. Surprisingly, the SPL group produced torque values that were not different than the SOS group in the last two sets, illustrating that preactivation at longer muscle lengths does not significantly increase peak torque production for the majority of the stretch-shortening cycle (Fig. 10).

DISCUSSION

The purpose of this study was to alter the timing of activation and starting muscle length before repetitive stretch-shortening cycles in an *in vivo* rabbit model and to measure the subsequent effects on fiber strain, peak force and muscle injury (7, 16, 22, 50). Traditionally, variables that influence muscle strain injuries have been measured in *in vitro* or *in situ* single-stretch protocols, whereby peak force and fiber strain have been studied as potential contributors to the muscle strain

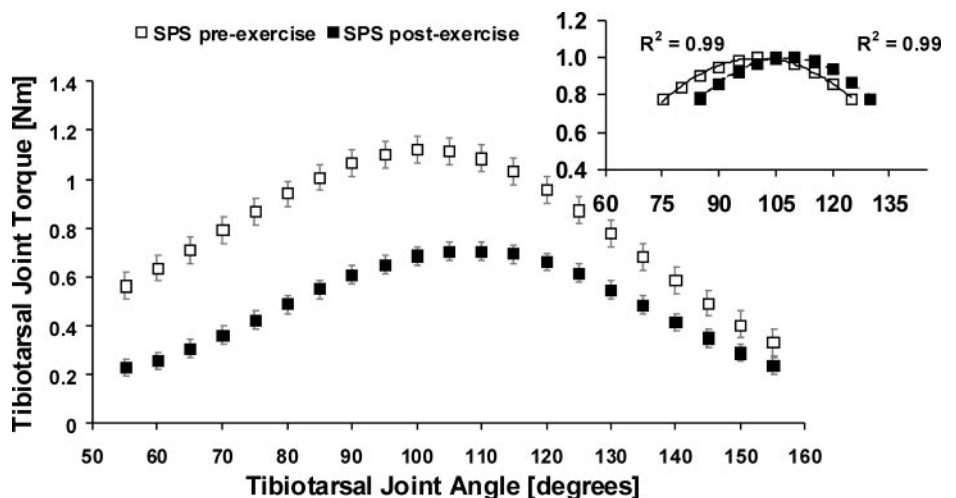


Fig. 7. Preexercise (\square) and postexercise (\blacksquare) torque-joint angle relationship for the SPS group. Note the significant shift of peak joint torque production to a greater joint angle and a change in the shape of the relationship after the exercise bout. *Inset*: normalized values $>75\%$ peak torque for comparing preexercise (\square) and postexercise (\blacksquare) values in each group were fitted with a second order polynomial, depicting the rightward shift in peak joint torque production after exercise ($+7.9 \pm 1.8^\circ$).

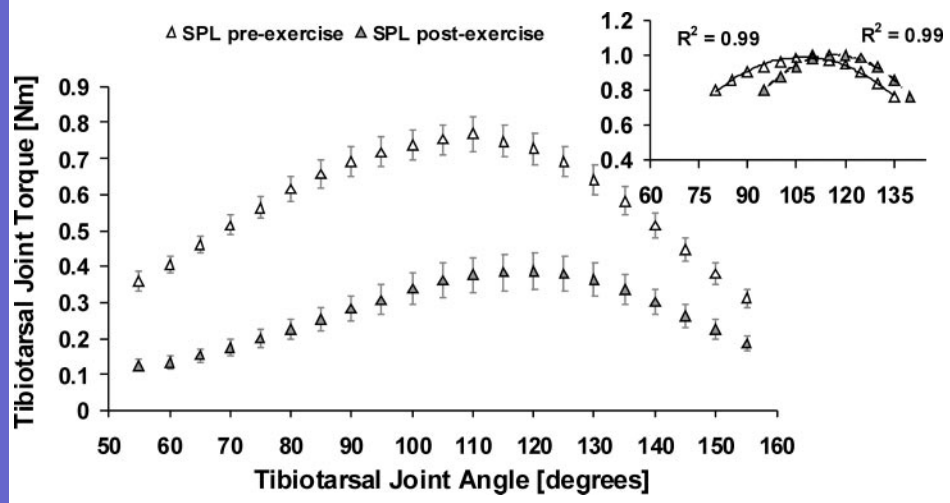


Fig. 8. Preexercise (Δ) and postexercise (\blacktriangle) torque-joint angle relationship for the SPL group. Note the significant shift of peak joint torque production to a greater joint angle and a change in the shape of the relationship after the exercise bout. *Inset*: normalized values $>75\%$ peak torque for preexercise (Δ) and postexercise (\blacktriangle) values in each group were fitted with a second order polynomial, depicting the rightward shift in peak joint torque production after exercise ($+10.9 \pm 1.0^\circ$).

injury etiology. In an effort to keep our protocol as physiologically representative as possible (22), we used a physiological range of motion in this study ($\sim 5\%$ MTU strain) and therefore needed to subject the muscles to a greater number of repetitive stretch-shortening cycles to induce injury (16, 23, 56, 57). Here, after repetitive lengthening contractions *in vivo*, muscle injury, as assessed by three different means, was significantly increased by altering activation timing at short lengths or increasing the starting length before the cycles.

Peak torque in the angle-torque relationship was produced at longer muscle lengths after all three exercise protocols (Fig. 6–8); however, the difference in the magnitude of the rightward shift was relatively small after exercise at short muscle lengths ($7.7 \pm 1.9^\circ$ in the SOS group, and $7.9 \pm 1.8^\circ$ in the SPS group) and was not significantly increased after exercise at long lengths ($10.9 \pm 1.0^\circ$ in the SPL group). Traditionally, the rightward shift in the FLR has been used as an indicator of muscle injury magnitude, with a greater injury resulting in a greater rightward shift (55). Recently, we showed that the shift in the FLR after lengthening contractions may not be sensitive enough to determine injury at short muscle lengths, where fatigue effects may confound the shift (11). Therefore we also quantified muscle injury by the difference in the reduction in joint torque between the three exercise groups.

To maintain a consistent metabolic cost during exercise bouts, activation duration was kept at 500 ms for all three protocols. Although there is evidence of a length dependence for fatigue in mammalian skeletal muscle, whereby exercise at long muscle lengths exhibits less fatigue compared with short lengths, there is some dispute as to whether isometric contractions at short muscle lengths (46, 47) exhibit greater fatigue than corresponding isometric contractions at optimal lengths (41). Here, the two groups used at the short muscle lengths exhibited significantly different torque decrement based on timing of activation, indicating a disparate effect of the activation timing on muscle damage, as fatigue is assumed to be similar in these two groups at identical exercise lengths. In addition, the dynamic, active lengthening contractions performed in the present study included the optimal tibiotarsal joint angle in the exercise range of motion in all groups. Also, if the two groups that were exercised at short muscle lengths exhibited greater fatigue compared with the groups exercised at long lengths, that would only exacerbate the difference in injury measured between the short and long length groups. Furthermore, the trend toward peak force production at longer muscle lengths, as well as a narrowing of the torque-joint angle relationship after exercise, further confirms that exercise at longer lengths resulted in greater subcellular damage compared

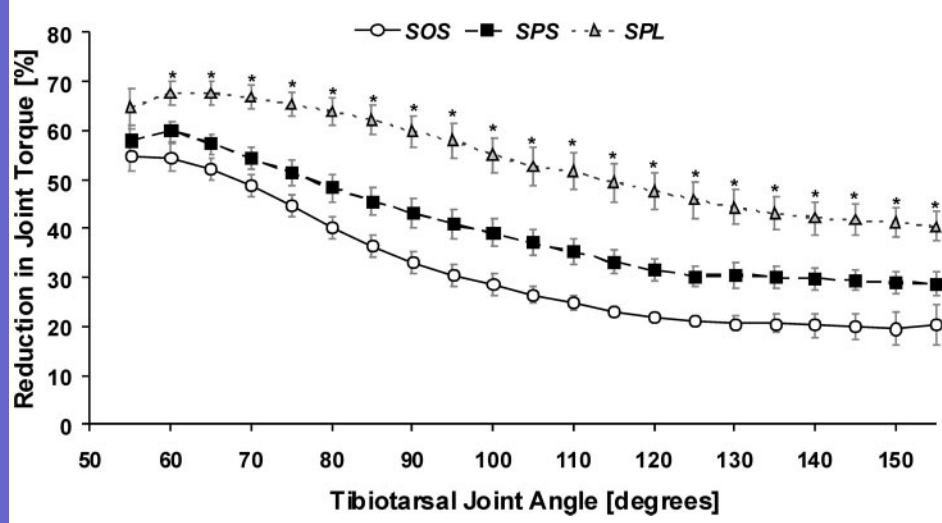
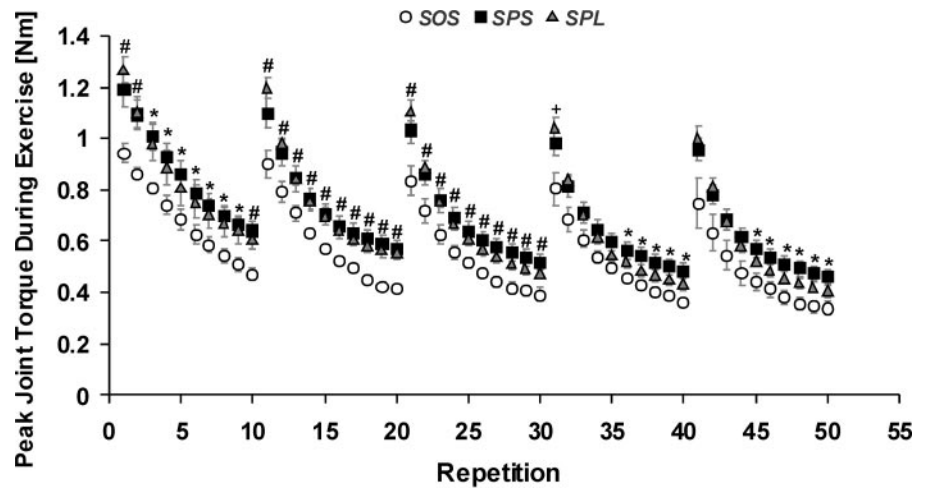


Fig. 9. Reduction in peak joint torque at all 21 tibiotarsal joint angles for the SOS (\circ), SPS (\blacksquare), and SPL (\blacktriangle) groups. Values are expressed as a percentage reduction from the preexercise joint-torque at the corresponding angle. Preactivation at short muscle length before stretch as well as increasing starting muscle length before stretch both resulted in significantly greater reduction in peak joint torque production at all tibiotarsal joint angles $>55^\circ$. *Significantly different reduction in postexercise isometric joint torque between all 3 groups at the joint angle indicated.

Fig. 10. Mean peak joint torque produced during the lengthening contractions for all 50 repetitions in the SOS (\circ), SPS (\blacksquare), and SPL (\blacktriangle) groups. Preactivation at short and long lengths resulted in a significantly greater torque production compared with exercise without preactivation. However, increasing muscle length before exercise (SPL) never increased joint torque compared with preactivation at short lengths (SPS). *SPS torque significantly greater than SOS torque; +SPL torque significantly greater than SOS torque; #SPS and SPL torque greater than SOS torque.



with exercise at short lengths (45). Therefore, we surmised that the significant differences in peak isometric torque values postexercise were due to injury and not fatigue (34).

We observed a significant difference in the reduction of isometric joint torque at all joint angles $>55^\circ$ between the three groups (Fig. 9), indicating that force production was affected at nearly all muscle and sarcomere lengths within the physiological range. The greatest reduction in peak joint torque observed was at short muscle lengths. Similar results have been reported previously after lengthening contraction induced injury and may be explained by damage to calcium handling structures (11, 16). Previously, we have measured a progressive increase in compliance of the contractile element during repeated stretch-shortening cycles (12), which may also contribute to the greater loss of force at short compared with long muscle lengths (42).

The reduction in peak isometric joint torque after lengthening contractions has been proposed to be the most sensitive means of quantifying muscle injury (44). Peak joint torque at optimal muscle length was decreased by 27% in the SOS group. This value increased to 37% by simply altering activation timing in the SPS group, while maintaining a consistent 5% muscle strain. In a previous study, it had been reported that the alteration of activation timing changed muscle injury magnitudes after three stretch-shortening cycles of 30% muscle strain *in vitro* (50). Here, we were able to produce muscle injury through repetitive stretch-shortening cycles using small magnitude muscle strains, within the physiological capabilities.

By increasing the length of the muscle before contraction (SPL), peak joint torque was reduced to a greater extent (50% of the preexercise value) after the stretch-shortening cycles, compared with those using short muscle lengths (SOS, SPS). Although muscle length has been proposed as a key variable in strain induced muscle injury, much of this work has been performed *in vitro* (23, 52, 58) or *in situ* (27, 55) by single or multiple stretch protocols, often utilizing large magnitudes of muscle stretch beyond the physiological range (27). Few studies have assessed the influence of starting muscle length on muscle injury *in vivo* (16, 39, 43), and only one has utilized a similar approach to the one used here (16). Using *in vivo* rat dorsiflexors, Cutlip et al. (16) found significantly greater muscle injury after 70 stretch-shortening contractions at long com-

pared with short muscle lengths. Because this study focused on the time-dependent effects of muscle injury, no attempt was made to explain why exercise at longer muscle lengths resulted in greater injury. Recently, muscle injury after repetitive lengthening contractions within a physiological range has been associated with the peak forces produced (23). However, as we have shown here, peak joint torque was significantly less for the majority of the stretch-shortening cycles at long muscle lengths compared with the corresponding peak torque at short muscle lengths. It is notable, however, that the rate of decay of joint torque appears to be greatest during exercise at long muscle lengths (Fig. 10), possibly indicating an increased rate of damage as exercise progressed.

In this study, preactivation of the muscle before stretch at short muscle lengths resulted in significantly greater peak joint torque production during the majority of the 50 stretch-shortening cycles compared with the SOS group (Fig. 10). This is in agreement with others who have preactivated the muscle in an effort to increase force production before stretch (34), but the first to show the difference across repetitions. Here, preactivating the muscle at short lengths resulted in both greater peak joint torque production and subsequently greater muscle injury, as defined by the decrease in isometric torque production at optimal length. In contrast, increasing the starting length of the muscle before stretch resulted in greater muscle injury, but it did not result in greater peak joint torque being produced during exercise, implying either that the mechanism of injury is different between the groups or that another variable may have a greater influence.

Fiber strains have never been directly measured during *in vivo*, controlled cyclic stretch-shortening cycles before. Although Lieber and Fridén (34) found fiber strain to be the best predictor of the observed muscle injury after repetitive lengthening contractions, fiber strains were estimated and assumed to be constant throughout 900 stretch-shortening cycles. However, in their study, increased fiber strain was achieved by a greater magnitude and rate of muscle stretch. Although greater muscle excursion has been shown to result in greater muscle injury (49), the direct association between injury, fiber dynamics and muscle dynamics can be tenuous, as we have shown here. In this study, we maintained an identical mechanical strain to the MTU during all three exercise protocols. By applying a preactivation before stretch at short muscle lengths,

fiber shortening and joint torque were significantly increased (Fig. 2). However, at long muscle length, active strain was significantly increased compared with active strain during exercise at short muscle lengths (Fig. 3), but torque values remained unaffected by increasing muscle length. Therefore, although active strain has been proposed to be the best predictor of muscle strain injury (34), it may not hold true for exercise at all muscle lengths, because peak torque was significantly increased only at short lengths, but active fiber strains were only greater at the long muscle length.

Although Lieber and Fridén measured muscle strain and estimated fiber length (34), we measured fiber length directly. By defining active strain as the magnitude of fiber stretch during the application of stimulation, active strain did not include the greatest fiber length change, as this occurred at the cessation of muscle stimulation. The significant increase in fiber strains observed at short muscle lengths using preactivation occurred at the cessation of external activation of the muscle. Although activation ceased before the end of muscle stretch, force was still produced by the muscle, albeit decreasing rapidly. This drop in force as the muscle approached the end range of stretch resulted in relaxation strains that were over 100% greater than those obtained without preactivation (Fig. 4).

Together, active strain and relaxation strain add up to what we have defined as maximum fiber strain (Fig. 1). At short muscle lengths active strain was not significantly increased with preactivation, although relaxation strain was. This may be due to the compliance at short muscle lengths, which allowed for greater active fiber shortening in the preactivated (SPS) compared with the normal (SOS) group. At the long length, the increased stiffness of the MTU prohibited great active shortening and therefore limited the amount of energy that could be stored and released within the MTU. Overall, maximum fiber strain was significantly increased with preactivation as well as increased starting muscle length, coincident with muscle injury. However, the manner in which maximum strain increased was different between the groups: first, at the short length, preactivation allowed for greater active shortening, no change in active strain, and greater relaxation strain; and, second, at the long muscle length, active shortening was limited, resulting in greater active strain and no change in relaxation strain. Together these active and relaxation strains result in a significantly greater maximum strain at the short muscle length owing to changes in the relaxation strain with preactivation and a greater maximum strain at the long muscle length owing to changes in active strain magnitude.

One limitation to this study was the method used to alter the timing of activation. To effectively limit the effects of fatigue across groups, we chose to use an activation of identical duration (500 ms) for all three groups. Therefore the timing of activation during the stretch-shortening cycles resulted in a shift of activation by 100 ms, causing relaxation strains to occur during passive plantar flexion in two of the three groups studied (SPS and SPL). It is possible that the duration of the activation period relative to the stretch duration may produce the differences observed between the groups and that the timing of activation cessation may be another important variable that must be considered in muscle injury research.

In summary, muscle strain injuries can be insidious, and the culmination of small mechanical alterations within the muscle

may result in sudden pain and loss of function. This is the first study to show that a muscle subjected to repeated stretch-shortening cycles of constant MTU excursion exhibits significantly different joint torque and fiber strains when the timing of activation or starting muscle length are changed. These results lend support to a mechanism of strain induced muscle injury as proposed by Best and Garrett (7), whereby a “poorly understood neuromuscular coordination pattern” may contribute to muscle strain injuries. Therefore, we conclude that activation timing and muscle length before stretch may influence muscle injury by significantly increasing fiber strain magnitude and that fiber dynamics are more important variables than MTU dynamics or muscle force in influencing the magnitude of muscle damage.

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