Impact of muscle length during stretch-shortening contractions on real-time and temporal muscle performance measures in rats in vivo

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Cutlip, R. G., K. B. Geronilla, B. A. Baker, M. L. Kashon, G. R. Miller, and A. W. Schopper. Impact of muscle length during stretch-shortening contractions on real-time and temporal muscle performance measures in rats in vivo. *J Appl Physiol* 96: 507–516, 2004. First published October 10, 2003; 10.1152/japplphysiol.00046. 2003.—The objective of the present study was to investigate the impact of muscle length during stretch-shortening cycles on static and dynamic muscle performance. Animals were randomly assigned to an isometric (control, Con, $n = 12$), a short-muscle-length (S-Inj, 1.22–2.09 rad, $n = 12$), or a long-muscle-length (L-Inj, 1.57–2.44 rad, $n = 12$) group. The dorsiflexor muscles were exposed in vivo to 7 sets of 10 stretch-shortening contractions (conducted at 8.72 rad/s) or 7 sets of isometric contractions of the same stimulation duration by using a custom-designed dynamometer. Performance was characterized by multipositional isometric exertions and positive, negative, and net work before exposure, 6 h after exposure, and 48 h after exposure to contractions. Real-time muscle performance during the stretch-shortening cycles was characterized by stretch-shortening parameters and negative, positive, and net work. The S-Inj group recovery (force difference) was similar to the Con group force difference at 48 h, whereas the L-Inj group force difference was statistically greater at 1.39, 1.57, and 1.74 rad than the Con group force difference ($P < 0.05$). Negative work ($P < 0.05$) and net work ($P < 0.05$) were statistically lower in the S-Inj and L-Inj groups than in the Con group 48 h after exposure to contractions. Of the real-time parameters, there was a difference in cyclic force with treatment during the stretch-shortening cycles ($P < 0.0001$), with the L-Inj group being the most affected. Thus longer ranges of motion result in a more profound isometric force decrement 48 h after exposure to contractions and in real-time changes in eccentric forces.

muscle injury; dorsiflexor muscles; cycle work

IT IS WELL ACCEPTED THAT EXPOSURE to eccentric contractions results in skeletal muscle damage in humans (28, 32) and animals (10, 16, 40, 41), whereas isometric and concentric muscle actions do not (33). Eccentric contractions result in ultrastructural damage immediately after exposure (11) and 1–3 days after exposure (14, 25). The extent of histological damage is difficult to quantify by light or electron microscopy, because only single sarcomeres or small groups of scattered sarcomeres are affected. The ultimate result of eccentric contractions is the inability of muscle to produce force and work. Studies have shown that the functional loss of isometric force is the best indicator of the totality of damage (10, 30, 39). Mechanical factors, such as peak force (13, 25), average force (4), work during the stretch (16), strain (21), and fiber length (13, 16), have been found to affect the magnitude of contraction-induced injury. The magnitude of the force deficit has been predicted by the following methods: *1*) work done during the stretch when initiated from optimal length (4, 25), *2*) initial length and work during the stretch when not initiated at optimal length (16), or *3*) peak force and initial length (13). Indeed, several studies have shown that stretches performed at longer ranges of motion or fiber length have resulted in larger isometric force deficits in humans (29) and animals (13, 16, 44).

Work during the eccentric contraction (negative work) has been shown to be well correlated with the isometric force deficit after a single eccentric contraction ($r^2 = 0.7–0.99$) (16, 25). Although the findings were informative, the length perturbation in these studies was beyond the normal physiological range of the target muscle. However, muscles stretched within the physiological range have required more than one repetition to produce injury (38, 41, 42). Studies of repetitive eccentric muscle actions in the physiological range may have more external validity than single-stretch models. Because the work absorbed during a stretch has been shown to be well correlated with the isometric force deficit, it would be informative to quantify the change in negative work during repetitive eccentric contractions to assess the impact of an eccentric protocol on muscle injury (evaluated by an isometric force deficit). The effect of injurious eccentric contractions on work produced during muscle shortening (positive work) should also be assessed (5, 34). A viable method to study eccentric and concentric muscle performance simultaneously in the context of muscle injury is via stretch-shortening cycles (reciprocal eccentric/concentric contractions). Exposure of dorsiflexor muscles to stretch-shortening cycles has resulted in significant myofiber damage similar to that evidenced in eccentric injury models (12). Using stretch-shortening cycles, one can isolate damage as assessed by a decrement in positive work vs. damage assessed by a decrement in negative work (34). Negative work has been shown to be more affected than positive work immediately after exposure to injurious stretch-shortening cycles in an isolated muscle preparation (34). However, no studies have examined whether the effect of exposure to eccentric contractions on concentric muscle performance (positive work) is different from eccentric (negative work) or isometric performance hours or days after exposure.

Stretch-shortening cycles have been studied in the context of human locomotion and athletic performance (1) and have been shown to produce muscle injury as a result of the eccentric component of the cycle (15). Natural muscle function is more often a stretch-shortening cycle; thus this approach provides an improvement over the traditional eccentric-only injury model (20). The pattern of length changes during stretch-shortening

cycles simulates in vivo function more accurately than the ramp stretches typically used (34). Also, single stretch-shortening cycles can be used to determine dynamic muscle function at time points of interest. This allows for a comparison between work (calculated from a single stretch-shortening cycle) and the more traditional isometric force as measures at different time points. This same approach can also be used for repetitive or injurious stretch-shortening cycles, in which work during each cycle and the change in work during an injurious exposure can be quantified. No studies have investigated the change in concentric (positive work) and eccentric (negative work) function before and after or during an injurious exposure and their relation to the magnitude of injury as assessed by an isometric force deficit. In addition, decay in peak eccentric forces, change in magnitude and decay of isometric force preceding each stretch, and decay in force enhancement during each stretch during an injurious exposure of stretch-shortening cycles (real-time changes) and their relation to the magnitude of muscle injury (assessed by an isometric force deficit) have not been investigated.

Our purpose was to investigate the effect of muscle length during an injurious stretch-shortening exposure on changes in force and work during and after the protocol. This would allow *1*) comparison of isometric force decrement and changes in work output at selected time points before and after injurious exposure, *2*) comparison of real-time changes in force and work during the injurious stretch-shortening cycles, and *3*) comparison of eccentric force deficits during injurious stretch-shortening cycles with isometric force deficits due to injury. Using an in vivo muscle preparation and custom-designed dynamometer (6), we studied the effect of two different ranges of motion on stretch-shortening cycle-induced muscle injury. We tested the specific hypothesis that repetitive stretch-shortening cycles conducted at a longer muscle length will result in a larger isometric force deficit than stretch-shortening cycles at a shorter muscle length.

We also hypothesized that stretch-shortening cycle muscle damage will have a greater effect on negative work than on positive work. In addition, we hypothesized that changes in real-time muscle performance would be most affected by stretch-shortening cycles conducted at a longer muscle length and would be predictive of the magnitude of isometric force deficit.

METHODS

Animal Handling

Male Sprague-Dawley rats [$n = 36$, 446 ± 32 (SD) g, 12 wk of age] were housed in an American Association for Accreditation of Laboratory Animal Care-approved animal quarters, with temperature and light-dark cycle (dark cycle from 7 AM to 7 PM) held constant for all animals and food and water provided ad libitum. After 1 wk of acclimatization, all animals were subjected to a standardized experimental protocol approved by the National Institute for Occupational Safety and Health Animal Care and Use Committee before the experimental protocols were conducted (Table 1). Animals were randomly assigned to a control (Con, $n = 12$), a short-muscle-length injury (S-Inj, 1.22–2.09 rad, $n = 12$), or a long-muscle-length injury (L-Inj, 1.57–2.44 rad, $n = 12$) group.

Experimental Setup

Rats were tested on a custom-built rodent dynamometer (5). A Labview-based virtual instrument was developed that governed a data acquisition board (model PCI-MIO-16XE-10, National Instruments) and a motion controller (Unidex 100, Aerotech, Pittsburgh, PA) for precise control of a brushless direct-current servomotor (model 1410 DC, Aerotech) and a muscle stimulator (model SD9, Grass Medical Instruments, Quincy, MA). The software also acquired and stored position, force, and velocity data in real time as described below. A small animal anesthetic system (Surgivet Anesco) was used to anesthetize rats with isoflurane gas. On the basis of contractile performance, stable ventilatory rates, and fast induction and recovery times, this class of anesthetic agents is an appealing choice for an acute or a long-term study examining skeletal muscle function (18). First, rats

Table 1. *Experimental procedure*

Step No.	Group		
	S-Inj ($n = 12$)	L-Inj ($n = 12$)	Con ($n = 12$)
1	Multipositional isometric, 1.22–2.44 rad in 0.175-rad increments	Multipositional isometric, 1.22–2.44 rad in 0.175-rad increments	Multi-positional isometric, 1.22–2.44 rad in 0.175-rad increments
2	1 Stretch-shortening contraction at 1.22–2.44–1.22 rad	1 Stretch-shortening contraction at 1.22–2.44–1.22 rad	1 Stretch-shortening contraction at 1.22–2.44–1.22 rad
3	7 Sets of 10 continuous stretch-shortening contractions at 1.22–2.09–1.22 rad	7 Sets of 10 continuous stretch-shortening contractions at 1.57–2.44–1.57 rad	7 Sets of isometric contractions at 1.57 rad
4	Multipositional isometric, 1.22–2.44 rad in 0.175-rad increments	Multipositional isometric, 1.22–2.44 rad in 0.175-rad increments	Multipositional isometric, 1.22–2.44 rad in 0.175-rad increments
5	1 Stretch-shortening contraction at 1.22–2.44–1.22 rad	1 Stretch-shortening contraction at 1.22–2.44–1.22 rad	1 Stretch-shortening contraction at 1.22–2.44–1.22 rad
6	Recovery: 6 and 48 h	Recovery: 6 and 48 h	Recovery: 6 and 48 h
7	Multipositional isometric, 1.22–2.44 rad in 0.175-rad increments	Multipositional isometric, 1.22–2.44 rad in 0.175-rad increments	Multipositional isometric, 1.22–2.44 rad in 0.175-rad increments
8	1 Stretch-shortening contraction at 1.22–2.44–1.22 rad	1 Stretch-shortening contraction at 1.22–2.44–1.22 rad	1 Stretch-shortening contraction at 1.22–2.44–1.22 rad
9	Animals killed: 6 and 48 h	Animals killed: 6 and 48 h	Animals killed: 6 and 48 h

To characterize isometric and dynamic performance of dorsiflexor muscles in an intact state, multipositional isometric tests (300-ms duration each) were conducted at 2-min intervals over 1.22–2.44 rad in 0.17-rad increments. After a 2-min rest, a stretch-shortening cycle was performed at 8.72-rad/s over the same range of motion. Groups were then exposed to 70 stretch-shortening cycles at a short muscle length (S-Inj, 1.22–2.09 rad) or long muscle length (L-Inj, 1.57–2.44 rad), both at 8.72 rad/s, or an isometric control (con, same stimulation duration as the short and long range of motion groups) conducted at 1.57-rad ankle angle. Posttesting consisted of multipositional isometric testing and stretch-shortening cycle testing immediately after, 6 h after, and 48 h after exposure. Posttesting was conducted in the same fashion as pretesting.

were placed in an "induction" tank filled with a mixture of isoflurane gas and oxygen. Then each rat was placed supine on the heated *x-y*-positioning table of the rodent dynamometer, with an anesthetic mask placed over its nose and mouth. The knee was secured in flexion (at 1.57 rad) with a knee holder. The left foot was secured in the load cell fixture with use of a custom-built foot holder with the ankle axis (assumed to be between the medial and the lateral malleoli) aligned with the axis of rotation of the load cell fixture. Each animal was monitored during the protocol to ensure proper anesthetic depth and body temperature. The animal setup was similar to that described by Willems and Stauber (43) and Geronilla et al. (12).

Functional Testing

The joint position of the animal was defined by the angle between the tibia and the plantar surface of the foot. The angular position of the load cell fixture corresponded with angular position of the ankle. Vertical forces applied to an aluminum sleeve fitted over the dorsum of the foot were translated to a load cell transducer (Sensotec) in the load cell fixture. The force produced by the dorsiflexor muscles was measured at the interface of the aluminum sleeve and the dorsum of the foot. Platinum stimulating electrodes (Grass Medical Instruments) were placed subcutaneously to span the peroneal nerve. Activation of the electrical stimulator resulted in muscle contraction of the dorsiflexor muscle group. Stimulator settings were optimized on the basis of pilot studies to maximize dorsiflexor isometric force using a supramaximal stimulus. Muscle stimulation for all protocols was a 120-Hz square-wave pulse at 0.2-ms pulse duration and 4 V. To reduce the effect of fatigue, all electrical stimulation times were kept to a minimum with recovery times between stimulations (43).

Pre- and Postmultipositional Isometric Tests

A series of isometric contractions were measured on the dorsiflexor muscle group starting at an ankle angle of 1.22 rad and performed in 0.17-rad increments to ≤2.44 rad. Each isometric contraction was performed every 2 min by using a 300-ms stimulation duration in a fashion similar to that described by Davis et al. (7) and Willems and Stauber (43). The series of isometric contractions was performed before (pre) and immediately after (post) seven sets of 10 stretch-shortening contractions (S-Inj and L-Inj groups) or 7 isometric contractions (Con group) to evaluate the effect on force production (Fig. 1). After a recovery interval of 6 or 48 h, animals from the S-Inj,

L-Inj, and Con groups ($n = 6$ from each group at each recovery interval) were tested as described above for the pre- and posttests. Each series of isometric tests was performed 2 min before all single stretch-shortening tests.

Single Stretch-Shortening Cycle

A single stretch-shortening contraction was measured on the dorsiflexor muscle group 2 min before and after an injury or the isometric control protocol. This test was used to evaluate the muscle's ability to perform work during dynamic stretch-shortening. The stretch-shortening contraction was performed by activating the dorsiflexor muscles for 300 ms and then moving the load cell fixture from 1.22 to 2.44 rad at an angular velocity of 8.72 rad/s. The load cell fixture was immediately returned to 1.22 rad, also at 8.72 rad/s. Activation was continued for 300 ms after cessation of the movement. After a recovery interval of 6 or 48 h, animals from the L-Inj, S-Inj, and Con groups ($n = 6$ from each group at each recovery interval) were retested as described above.

Injury Protocol

Two groups were exposed to an injury protocol that consisted of stretch-shortening contractions. Each group received 70 stretch-shortening contractions performed at a range of motion of 1.22–2.09 rad (S-Inj) or 1.57–2.44 rad (L-Inj). The stretch-shortening contractions were performed by fully activating the dorsiflexor muscles for 100 ms and then moving the load cell fixture in the prescribed range of motion at a velocity of 8.72 rad/s, in a reciprocal fashion, for 10 oscillations (requiring 2.4 s because of motor ramp-up and ramp-down times). After 10 immediately successive oscillations were complete, the load cell fixture was stopped, and the dorsiflexor group was deactivated 300 ms later. The total stimulation time per set was 2.8 s. The 7 sets of 10 oscillations were conducted at 1-min intervals.

Isometric Control Protocol

The Con group was exposed to seven maximal isometric contractions conducted at 1-min intervals. During each contraction, the same stimulation parameters and duration used in the two injury groups were used to stimulate dorsiflexor muscles for 2.8 s at an ankle angle of 1.57 rad.

Data Analysis

Reciprocal stretch-shortening cycles. The experimental data for the seven isometric contractions (Con, $n = 6$) and seven sets of stretch-shortening contractions (L-Inj and S-Inj, $n = 12$) were initially analyzed, and it was determined that *sets 1, 3, 5,* and *7* represented the changes in mechanical behavior during the protocol. The data were processed to identify the parameters that characterized the mechanical responses of the dorsiflexor muscle group during repeated stretch-shortening contractions. Peak force (F_{peak}, defined as peak eccentric force), minimum force (F_{min}, defined by the isometric force preceding each stretch), average force (F_{mean}, obtained by calculating the average of muscle force values during the 100-ms eccentric phase of each oscillation), and cyclic force [$F_a = (F_{peak} - F_{min})$, defined by the force enhancement during each stretch] during the stretch-shortening contractions were evaluated (Fig. 2). These parameters were quantified for the first oscillation in each set to determine changes between sets. Group means of F_{peak}, F_{min}, F_a, and F_{mean} and the standard error of the mean of those parameters were calculated for each group. The experimental data from the first oscillate of *sets 1, 3, 5,* and *7* of the L-Inj and S-Inj groups were used to calculate negative work (integral of the force-position plot during the eccentric phase of the cycle), positive work (integral of the force-position plot during the concentric phase of the cycle), and net work (difference between calculated negative work and positive work) during the injury protocol. Work was calculated as described by Ettema (9), Stevens and Faulkner (35),
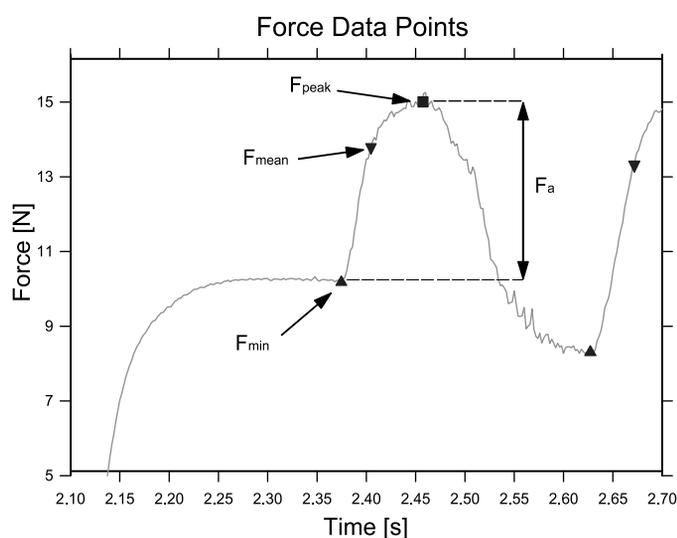

Fig. 1. Evaluation of stretch-shortening cycle parameters [peak force (F_{peak}), minimum force (F_{min}), cyclic force ($F_a = F_{peak} - F_{min}$), and average force (F_{mean})] for *sets 1–7*.
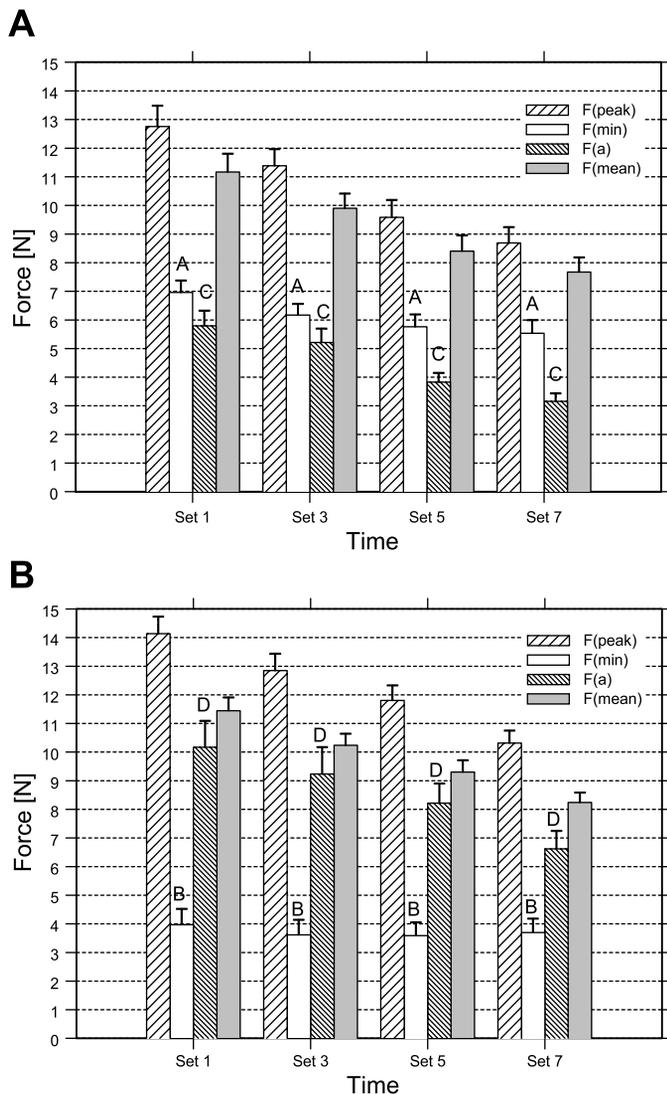
A

B

Fig. 2. F_{mean}, F_a, F_{peak}, and F_{min} as a function of set for the long muscle length (L-Inj) group (*A*) and the short muscle length (S-Inj) group (*B*). [A]Statistical difference between L-Inj and S-Inj for F_{min}. [B]Statistical difference between L-Inj and S-Inj for F_{min}. [C]Statistical difference between L-Inj and S-Inj for F_a. [D]Statistical difference between L-Inj and S-Inj for F_a. Statistical difference was reported at the 0.05 level.

and Stevens (34). Group means of positive, negative, and net work and the standard error of the mean of those parameters were calculated for the L-Inj and S-Inj groups.

Single stretch-shortening cycle. The experimental data from each stretch-shortening cycle were used to calculate negative work, positive work, and net work as described for the injury protocol. Negative work, positive work, and net work were calculated for each animal before and 6 and 48 h after the test. Group means of positive, negative, and net work and the standard error of the mean of those parameters were calculated for each group. Additionally, the pure stretch-shortening cycle work was calculated by subtracting the baseline isometric force of each animal from the forces generated over the range of motion during each stretch-shortening cycle. Negative work, positive work, and net work were then calculated for each animal before and 6 and 48 h after the test to quantify the effect of changes in baseline isometric force on stretch-shortening cycle work.

Statistical analysis methodology. Statistical analyses were conducted by using SAS version 8 (SAS Institute, Cary, NC). Mixed-

model analyses of variance with repeated measures were utilized for the analysis of multipositional isometric force measures and for the force and work measures (positive, negative, and net) recorded during the injury protocol. The difference between preinjury and recovery forces was utilized as the response variable for the multipositional force measures. Calculated work measures and stretch-shortening cycle parameters were analyzed by using analyses of variance. Post hoc analysis where main effects or interactions were significant was subsequently performed by using Fisher's least significant difference test. Regression analyses were performed on temporal measures of net work, positive work, and negative work and their respective correlation to the difference between preinjury and recovery isometric forces (incorporating 6- and 48-h force deficits) at each ankle angle for the Con, S-Inj, and L-Inj groups ($n = 36$). In addition, changes in net work, positive work, and negative work of the Con, S-Inj, and L-Inj groups ($n = 36$) were analyzed with respect to time at 6 and 48 h for their respective correlation to preinjury and recovery isometric forces at the corresponding time point. Regression analyses were also performed on the changes in the temporal measures of net work, positive work, and negative work at 6 and 48 h of the S-Inj and L-Inj groups ($n = 24$) and their correlation to real-time changes in those respective parameters from *set 1* to *set 7*.

RESULTS

Real-Time Changes in Stretch-Shortening Cycle Parameters

The changes in peak force from *set 1* to *set 7* during the seven sets of stretch-shortening cycles were not statistically different with treatment ($P = 0.813$; Fig. 2). The changes in mean force during the stretch-shortening cycles also did not differ with treatment ($P = 0.265$; Fig. 2). However, there was a difference in cyclic force with treatment during the stretch-shortening cycles ($P < 0.0001$; Fig. 2). The S-Inj group showed a decrease in cyclic force of 34% from *set 1* to *set 7,* whereas the L-Inj group exhibited a 47% decrease. The minimum force also decreased differently with treatment ($P < 0.0001$; Fig. 2).

Real-Time Changes in Stretch-Shortening Work Parameters

The real-time changes in negative work, positive work, and net work did not differ with treatment ($P = 0.878$, 0.879, and 0.652, respectively) but did change significantly from *set 1* to *set 7* ($P = 0.001$, 0.04, and 0.001, respectively; Fig. 3). Closer inspection of the data revealed that negative work in the L-Inj group decreased 34% during the protocol, whereas negative work in the S-Inj group decreased 29%. Positive work in both groups decreased by ~20%, whereas net work decreased by 65% and 50% for the L-Inj and S-Inj groups, respectively (Fig. 3).

Multipositional Isometric Forces

The isometric preprotocol forces in the range of motion tested (1.22–2.44 rad) for all three groups were not statistically different. The post-6-h forces of the Con and L-Inj groups recovered to ~66–82% of the preprotocol forces at all angles (Fig. 4, *A* and *B*), whereas those of the S-Inj group recovered to only 50–60% of the preprotocol forces (Fig. 4*C*). At 48 h after exposure, the post-48-h forces in the Con group recovered to 95% of the preprotocol forces at all angular positions except 1.22 rad (Fig. 4*A*), whereas those in the S-Inj group did not recover to the 95% level at angular positions of 1.57–2.44 rad
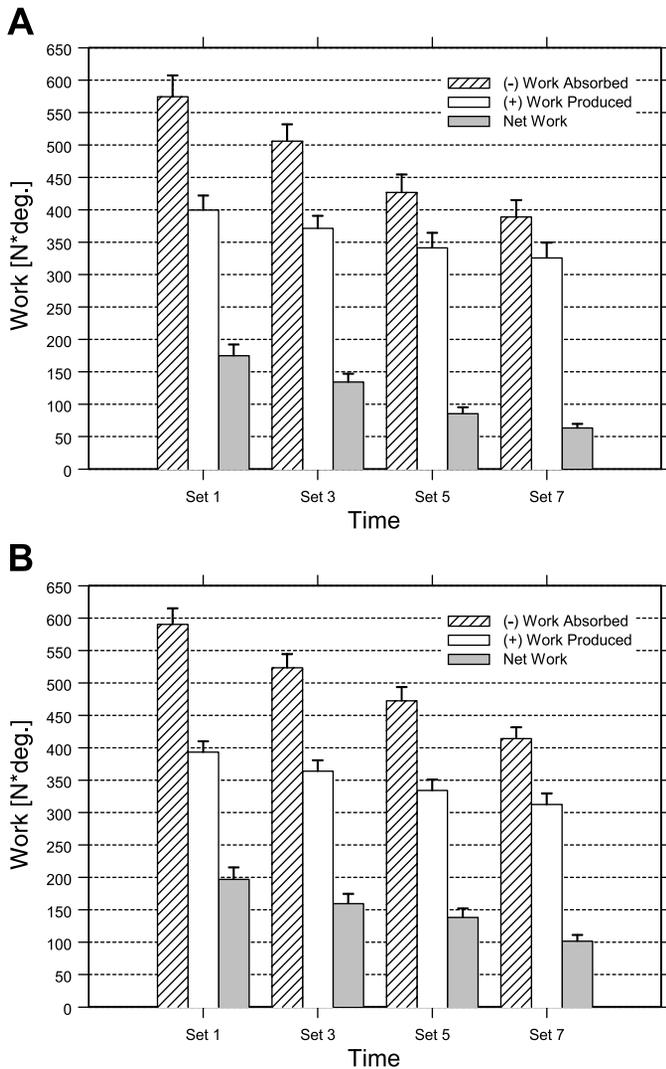
Fig. 3. Stretch-shortening cycle work parameters net work, negative work, and positive work as a function of set for the L-Inj group (*A*) and the S-Inj group (*B*). There was no statistical difference between L-Inj and S-Inj for any of the work parameters. Statistical difference was reported at the 0.05 level.

(Fig. 4*C*). The L-Inj group also did not recover to the 95% level at angular positions of 1.39–2.44 rad (Fig. 4*B*). The difference between the preprotocol and post-6-h and between the preprotocol and post-48-h isometric forces at each angular position were calculated for all groups. The differences in the S-Inj and L-Inj group values were compared with the Con values (Fig. 5). The difference in the preprotocol and 6-h recovery forces of the S-Inj group were statistically greater than the Con force differences at 1.39–2.09 rad, whereas the L-Inj force difference was similar to the Con force difference at all angles except 1.22 rad (Fig. 5*A*). The 48-h recovery forces showed a pattern that was quite different from the 6-h recovery forces. The S-Inj group recovery (force difference) at 48 h was similar to the Con force difference (Fig. 5*B*). In contrast, the difference in the preprotocol and 48-h recovery forces at 1.39, 1.57, and 1.74 rad was statistically greater for the L-Inj than for the Con group ($P = 0.006$, 0.028, and 0.042, respectively; Fig. 5*B*).
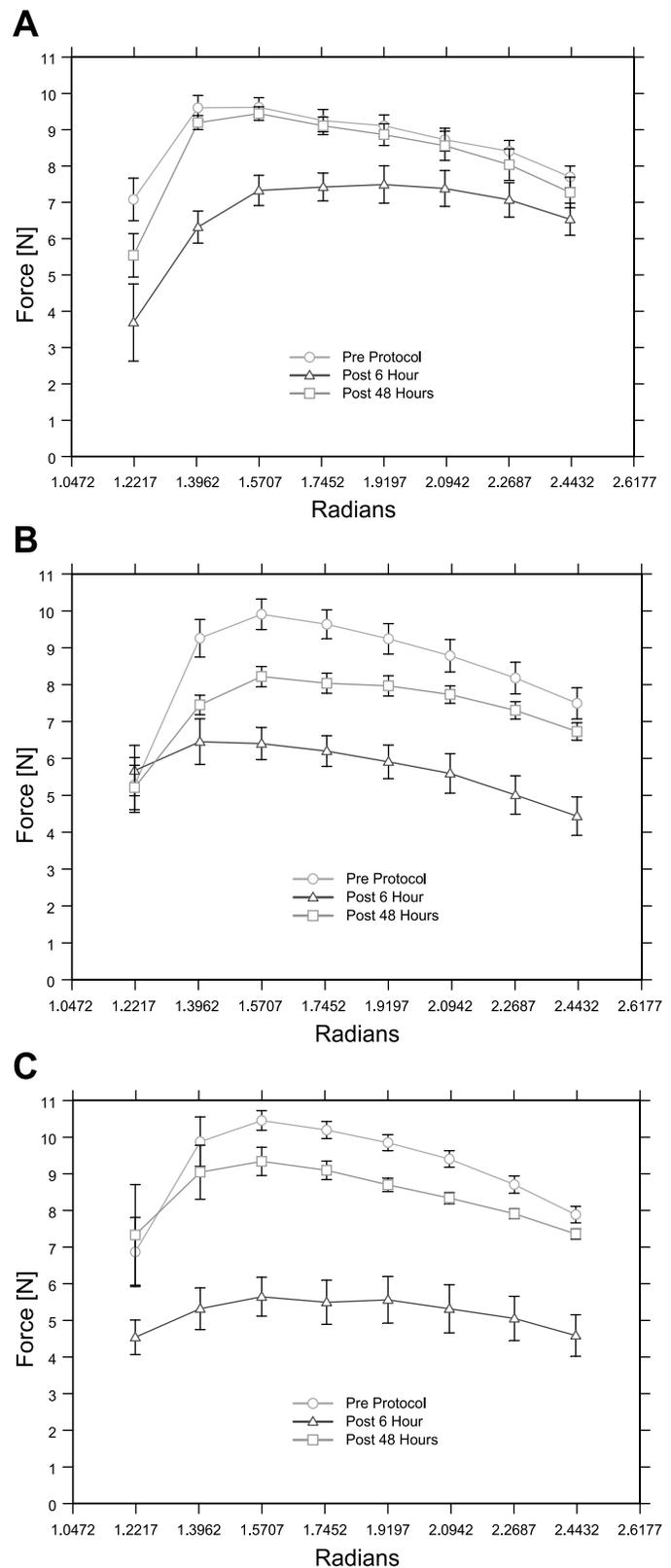

Fig. 4. Isometric force as a function of ankle angle (multipositional isometric testing) at pretest, 6 h posttest, and 48 h posttest for isometric control (*A*), L-Inj (*B*), and S-Inj (*C*) groups. Values are means ± SE.
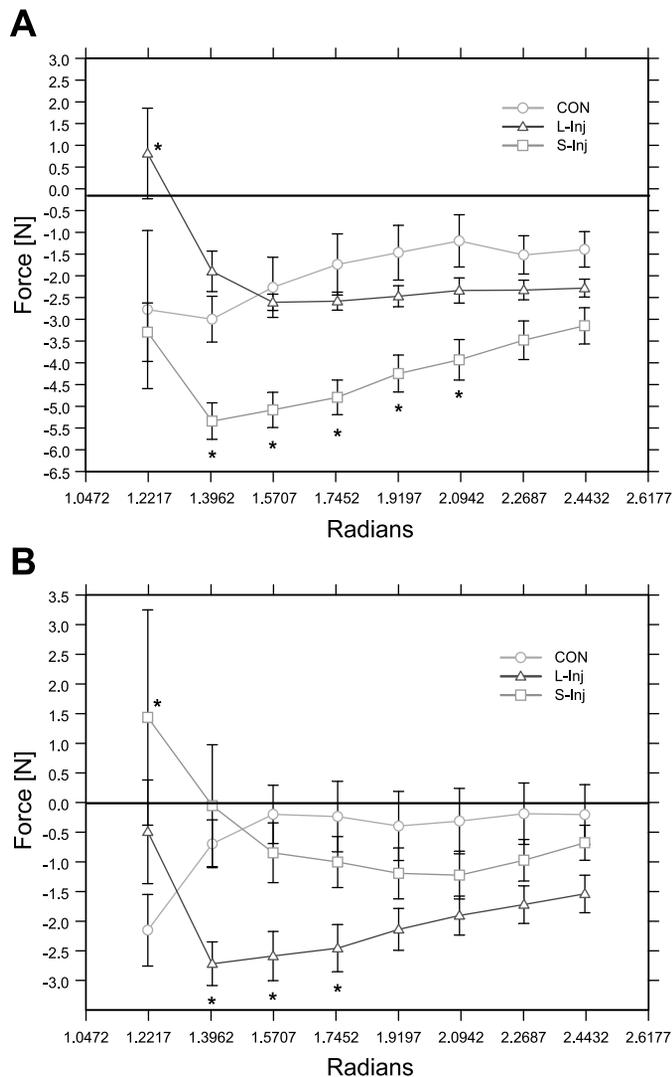
A

B

Fig. 5. Difference between preinjury and 6-h recovery forces (*A*) and between preinjury and 48-h recovery forces (*B*) for Con, L-Inj, and S-Inj groups as the response variable for multipositional force measures. Values are means ± SE. Analysis of the data indicated that the difference in the pretest and 6-h posttest isometric forces of the S-Inj group was statistically different from the Con force difference at ankle angles of 1.39–2.09 rad. Difference between pretest and 6-h recovery forces of the L-Inj group was statistically different from Con force difference at 1.22 rad. Difference in pretest and 48-h posttest isometric forces of the L-Inj group was statistically different from the Con force difference at ankle angles of 1.39–1.74 rad. Difference between pretest and 48-h recovery forces of the S-Inj group was statistically different from Con force difference at 1.22 rad. Statistical difference was reported at the 0.05 level.

Time-Dependent Changes in Negative Work, Positive Work, and Net Work

The time-dependent changes in positive work did not show a treatment-time interaction between the Con, S-Inj, and L-Inj groups ($P = 0.092$). Post hoc analyses indicated that the positive work of the Con group was not statistically different from that of the L-Inj group at 6 h after exposure ($P = 0.42$) but approached significance from positive work of the S-Inj group ($P = 0.051$; Fig. 6*A*). However, at 48 h after exposure, the S-Inj and L-Inj groups were not statistically different from the Con group ($P = 0.45$ and 0.44 respectively; Fig. 6*A*). In

contrast to temporal changes in positive work, negative work was affected by treatment ($P < 0.0001$). Negative work was significantly greater at 6 h after exposure in the Con group than in the S-Inj and L-Inj groups ($P = 0.0009$ and 0.001, respectively). At 48 h after exposure, negative work was statistically greater in the Con group than the S-Inj or L-Inj groups ($P = 0.019$ and 0.0001, respectively; Fig. 6*B*). The temporal changes in net work were similar to the changes in negative work. The treatment effect was significant ($P = 0.0001$). At 6 h after exposure, net work in the Con group was not different from that in the S-Inj group ($P = 0.09$) but was greater than in the L-Inj group ($P = 0.0069$). At 48 h after exposure, net work was statistically less in the S-Inj and L-Inj groups than in the Con group ($P = 0.0022$ and 0.0007, respectively; Fig. 6*C*). Net work of the S-Inj and L-Inj groups was not statistically different ($P = 0.66$; Fig. 6*C*). Subtraction of the baseline isometric force from the negative, positive, and net work calculations resulted in findings similar to those observed when work values were calculated with the baseline isometric force included. Although values of negative and positive work dropped 300 N·deg in magnitude, post hoc analysis indicated the same trends between groups at 6 and 48 h after exposure for negative work and net work (data not shown). Positive work at 6 h after exposure was significantly different for the L-Inj and S-Inj groups than for the Con group ($P = 0.043$ and 0.0453, respectively) but was not statistically different from the Con group at 48 h after exposure ($P = 0.758$ and 0.263, respectively). This was similar to the positive work findings with the baseline isometric force included, although the trend at 6 h after exposure was more apparent when the positive work was calculated with the baseline isometric force subtracted.

Relation Between Time-Dependent Changes in Negative, Net, and Positive Work and Isometric Force Deficit

Correlations between time-dependent changes in negative, positive, and net work vs. isometric force deficit at 6 and 48 h indicated that changes in negative and positive work were highly correlated with changes in isometric force over the same time. R^2 values ranged from 0.71 to 0.86 for negative work and from 0.71 to 0.76 for positive work at ankle angles of 1.40–2.44 rad (Table 2). They were not well correlated at an ankle angle of 1.22 rad. Changes in net work were not well correlated with changes in isometric force ($R^2 = 0.25$–0.40 at ankle angles of 1.40–2.44 rad; Table 2). When the correlations with time were analyzed, negative work ($R^2 = 0.53$–0.84) and positive work ($R^2 = 0.55$–0.64) were well correlated at 6 h (Table 2) and 48 h (negative work values ranged from 0.67 to 0.83, positive work values ranged from 0.57 to 0.76; Table 2) at ankle angles of 1.40–2.44 rad. Again, they were not well correlated at an ankle angle of 1.22 rad at 6 or 48 h. Net work was not well correlated with isometric force deficit at either time point (Table 2).

Relation Between Real-Time Changes in Negative, Positive, and Net Work and Time-Dependent Changes in Those Respective Parameters

Correlations between real-time changes in negative, positive, and net work vs. time-dependent changes in those parameters indicated that real-time changes in negative work from *set 1* to *set 7* were well correlated with time-dependent changes in

that parameter at 6 h ($R^2 = 0.61$) and 48 h ($R^2 = 0.69$; Table 3). Real-time changes in positive work were not as well correlated with temporal changes in that parameter at 6 h ($R^2 = 0.03$) and 48 h ($R^2 = 0.49$; Table 3). Real-time changes in net

A

B

C

Table 2. *Coefficient of determination of change in time-dependent variables vs. isometric force deficit of Con, S-Inj, and L-Inj groups*

	Ankle Angle, rad							
Variable	1.22	1.40	1.57	1.74	1.92	2.09	2.27	2.44
			6 and 48 h (n = 36)					
Negative work	0.1	0.71*	0.86*	0.81*	0.75*	0.74*	0.76*	0.73*
Positive work	0.25	0.71*	0.74*	0.76*	0.77*	0.72*	0.71*	0.74*
Net work	−0.08	0.25	0.40*	0.33*	0.26	0.28	0.31	0.25
			6 h (n = 18)					
Negative work	0.04	0.53*	0.84*	0.77*	0.67*	0.61*	0.55*	0.53*
Positive work	0.18	0.63*	0.62*	0.64*	0.64*	0.63*	0.56*	0.55*
Net work	−0.11	0.01	0.33	0.25	0.14	0.10	0.09	0.07
			48 h (n = 18)					
Negative work	−0.03	0.67*	0.83*	0.79*	0.75*	0.79*	0.79*	0.71*
Positive work	0.15	0.57*	0.64*	0.72*	0.76*	0.68*	0.63*	0.70*
Net work	−0.12	0.34	0.46	0.38	0.30	0.40	0.43	0.40

*Statistical significance at the 0.05 level.

work were also not well correlated at 6 h ($R^2 = 0.40$) but were well correlated at 48 h ($R^2 = 0.92$; Table 3).

DISCUSSION

Past investigations of eccentric contraction-induced muscle injury utilizing single stretch models have identified key mechanical factors such as work, strain (incorporating initial and final length), and initial length as causal components in the injury process (3, 4, 16, 24, 25). Although results from single stretch models have been informative about the causal factors in muscle injury, the target muscles were studied outside the normal physiological range. Because fibers were typically stretched to 50% beyond optimal fiber length, muscle injury could have occurred independent of muscle activation. Indeed, passive length perturbations outside the physiological range can result in significant strain injury (4). Thus it would be difficult to assess the contribution of passive vs. active muscle injury in those models. In other studies, more than one stretch within the physiological range was required to produce muscle injury (13, 38, 41, 42). Typically, peak force during the stretch was identified as the primary factor associated with the resultant isometric force deficit (13, 38). Our observation supports those findings: more than one repetition is required in vivo to result in muscle injury.

In our in vivo model, we observed that exposure to stretch-shortening cycles at longer muscle lengths did indeed result in a larger isometric force deficit than in the Con group after 48 h
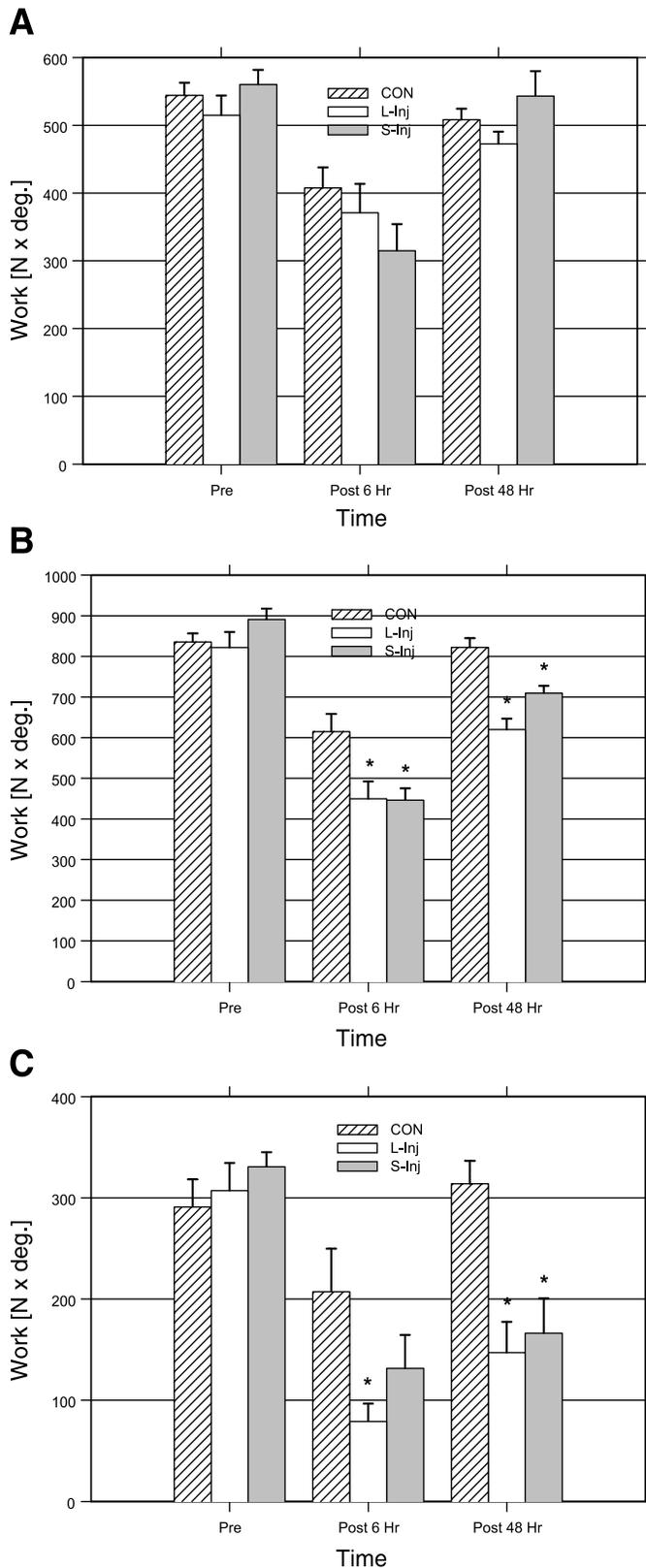
Fig. 6. Positive (A), negative (B), and net (C) work of Con, L-Inj, and S-Inj groups pretest, 6 h posttest, and 48 h posttest. A: positive work was not statistically different between Con group and L-Inj and S-Inj groups pretest, 6 h posttest, or 48 h posttest. B: negative work in the L-Inj and S-Inj groups was statistically different from negative work in the Con group 6 and 48 h posttest (indicated by *). C: temporal changes in net work were similar to changes in negative work. Treatment effect in net work was significant. For net work 6 h posttest, the Con group was not different from the S-Inj group but was different from the L-Inj group (indicated by *). For net work 48 h posttest, the S-Inj and L-Inj groups were statistically different from the Con group (indicated by *). Net work of the S-Inj and L-Inj groups was not statistically different. Values are means ± SE. Statistical difference was reported at the 0.05 level.

Table 3. *Coefficient of determination of change in real-time variables vs. time-dependent changes in those variables*

Variable	6 h	48 h
Negative work	0.61*	0.69*
Positive work	0.03	0.49
Net work	0.40	0.92*

*Statistical significance at the 0.05 level.

of recovery. This finding agrees with previous animal studies utilizing injury paradigms conducted outside the normal physiological range (3, 16, 44) and within the physiological range (13) and with human studies conducted within the physiological range (29). The dynamometry model was chosen over volitional studies for precise control of movement and muscle activation and accurate measurement of static and dynamic muscle forces. The dorsiflexor muscle group was chosen on the basis of many previous studies with mice (4, 10, 25, 40), rats (8, 12, 24, 37), and rabbits (2, 7, 21) and the pennate fiber architecture of the muscle group. Isometric force can be depressed for some time because of exposure of noninjurious isometric contractions (27) or injurious eccentric or stretch-shortening contractions (12, 17, 19, 23). Our results indicated that isometric forces of all three groups were depressed 6 h after exposure to isometric or stretch-shortening contractions. Although the isometric force decrement was greatest in the S-Inj group at 6 h, it is believed to be due to fatigue, because the Con group also exhibited an isometric force decrement at 6 h. This is not surprising, because it has been noted that exposure to noninjurious isometric contractions can depress isometric forces immediately after (22) and for days after exposure (27). In contrast, at 48 h after exposure, only the group exposed to stretch-shortening contractions at the longer muscle length exhibited isometric force depression statistically different from the Con group. The isometric force deficit was maximum at shorter muscle lengths (corresponding with smaller ankle angles) at 6 h (S-Inj) and 48 h (L-Inj) after exposure. This is similar to the results reported using rat soleus muscle bundles (45). One finding of interest was that isometric force deficits at the ankle angle of 1.2217 rad (70°) for the S-Inj group at 6 h and for the L-Inj group at 48 h were less than the force deficit at all other ankle angles for those groups. However, the isometric force deficit at 1.2217 rad was not well correlated with changes in negative or positive work, whereas correlations were significant for all other ankle angles. Thus changes in isometric performance at an ankle angle of 1.2217 rad were not reflective of changes in dynamic muscle performance, particularly positive and negative work. One explanation for the depression of isometric force in the Con group at 1.2217 rad at 6 and 48 h may be enhanced activation failure at short muscle lengths from failure of sarcolemmal action potential propagation in the t tubule network (31). The compromised electrical propagation at short muscle lengths has been shown in humans (31) and earlier in striated muscle fiber work (36).

Although work done during a single stretch was reported to be a good predictor of the degree of muscle damage (4, 25) and peak force was reported to be highly correlated with the degree of muscle damage in multiple-repetition models (13, 38), the change in work and the change in eccentric forces during an injurious protocol of repetitive contractions and the temporal response of negative and positive work have not been reported. The change in negative and positive work at 6 and 48 h was highly correlated with isometric force deficit at 6 and 48 h. This finding indicates that changes in dynamic muscle function due to exposure to isometric contractions or injurious stretch-shortening cycles reflect changes in isometric muscle function. One advantage to assessing dynamic performance changes in addition to isometric performance is the ability to distinguish whether the effect on eccentric muscle function is different from the effect of concentric muscle function after exposure to injurious or noninjurious muscle contractions. Our findings suggest that the ability of muscle to perform eccentric work (negative work) was significantly affected in both injury groups up to 48 h after exposure to injurious stretch-shortening cycles and that longer muscle length may exacerbate that effect. This is in agreement with earlier work conducted in mouse muscle in vitro (34), where eccentric work was affected immediately after exposure. In contrast, exposure to only isometric contractions did not affect the muscle's ability to perform eccentric work 48 h after exposure. Histological evidence supports the findings from eccentric work that as little as 70 stretch-shortening cycles can produce significant myofiber necrosis 48 h after exposure, whereas exposure to equivalent isometric contractions does not (12). In addition, exposure to injurious stretch-shortening contractions did not compromise the ability of muscle to perform concentric work (positive work) 48 h after exposure. Positive work for all groups was depressed 6 h after exposure because of fatigue, as also evidenced in isometric force tests, and all groups recovered 48 h after exposure. Stretch-shortening cycles also reduced the muscle's ability to perform net work. By 48 h after exposure, net work of the injured groups was still depressed, whereas exposure to isometric contractions did not result in any depression in net work. This result was not surprising, because net work is the difference between negative and positive work for a given cycle. In addition, the time-dependent changes in negative, net, and positive work were not affected by changes in isometric forces over the 48-h postexposure period. Although the magnitude of negative and positive work was reduced when the isometric baseline force was subtracted, the trends were quite similar. This finding was important, because changes in the pure stretch-shortening cycle work (as defined by subtracting the influence of baseline isometric force) were indicative of changes in dynamic muscle function. Specifically, negative work was affected 48 h after exposure, irrespective of changes in isometric forces. This indicates that pure eccentric muscle function was affected 48 h after exposure to injurious stretch-shortening cycles. This result was also reflected in the changes in net work, which by definition does not include influences of isometric forces, because it is the arithmetic difference between negative and positive work.

Thus mechanical measurements (isometric force and work) can be performed longitudinally at the appropriate time when the effects from fatigue (as assessed by an isometric control group) are ameliorated. In this way, noninvasive in vivo models are advantageous to the study of muscle injury. Although only the L-Inj group exhibited an isometric force deficit 48 h after exposure compared with the Con group, negative work was depressed in the S-Inj and L-Inj groups. This result suggests that the muscle's ability to produce eccentric work is

more affected than isometric muscle performance after an injurious bout of stretch-shortening contractions. Thus isometric muscle testing, although it is well correlated with dynamic muscle function, may not be as sensitive to muscle damage as changes in negative work. Also, isometric muscle testing alone cannot be used to infer the dynamic properties of muscle performance, whereas a single stretch-shortening cycle can.

In addition to temporal measures of muscle function, real-time measures of work and force parameters during injurious stretch-shortening contractions are informative about the injury process and can also be predictive of the temporal response of muscle performance after exposure to injury. In our study, the force parameter (F_a) that describes the force enhancement during stretch was affected more during stretch-shortening cycles at a longer muscle length. The different response in F_a between groups indicated that the muscle's ability to produce eccentric force was compromised more during injurious stretch-shortening cycles when conducted at longer fiber lengths (via a larger ankle angle). This finding is supportive of previous work conducted by Gosselin and Burton (13). This result was also reflected in the multipositional isometric performance 48 h after exposure. Real-time changes in negative work from *set 1* to *set 7* showed a pronounced decrement in both groups and a decrement in negative work at 48 h, also in both groups. Thus the real-time changes in negative work were well correlated with temporal changes at 6 and 48 h. This may suggest that change in negative work during the stretch-shortening cycles is due to injury, rather than fatigue, because we see this same reduction 48 h later, after the isometric control group has fully recovered its isometric force and dynamic performance (as assessed by the ability to perform work). Thus changes in real-time eccentric performance (assessed by changes in F_a and negative work) may be suggestive of injury. In contrast, positive work fully recovered in the S-Inj and L-Inj groups at 48 h but showed a decline in real time between *set 1* and *set 7*, thus resulting in a lower correlation. This may be due to the transient effects of fatigue that are present in real time and shortly thereafter (at 6 h) but are ameliorated as time passes. Real-time changes in net work, which incorporates positive and negative work, were not as well correlated at 6 h because of the effects of fatigue (represented by the positive work component) but were well correlated 48 h after the effects of fatigue were ameliorated, thus showing the contribution of negative work. Thus real-time changes in eccentric muscle performance are indicative of injury that will be reflected in dynamic and static performance measures temporally.

In summary, stretch-shortening cycles conducted at longer muscle lengths (via larger ankle angles) resulted in a larger isometric force deficit 48 h after exposure, which is supportive of earlier studies (13, 16, 26, 44). The larger decrements in negative and net work and less decrement in positive work during the injurious stretch-shortening cycles were reflected in the temporal work parameter decrements found in the single stretch-shortening cycle 48 h after exposure. Stretch-shortening cycles conducted at longer muscle lengths resulted in more profound real-time changes in force enhancement during the stretch-shortening cycles (F_a). This was similarly reflected in multipositional isometric muscle performance 48 h after exposure.

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