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Exercise following a short immobilization period is detrimental to tendon properties and joint mechanics in a rat rotator cuff injury model

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Abstract

Rotator cuff tears are a common clinical problem that can result in pain and disability. Previous studies in a rat model have shown enhanced tendon to bone healing with post-operative immobilization. The objective of this study was to determine the effect of post-immobilization activity level on insertion site properties and joint mechanics in a rat model. Our hypothesis was that exercise following a short period of immobilization will cause detrimental changes in insertion site properties compared to cage activity following the same period of immobilization but passive shoulder mechanics will not be affected. We detached and repaired the supraspinatus tendon of twenty-two Sprague-Dawley rats and the injured shoulder was immobilized post-operatively for 2 weeks. Following the immobilization period, rats were prescribed cage activity or exercise for 12 weeks. Passive shoulder mechanics were determined and following sacrifice, tendon cross-sectional area and mechanical properties were measured. Exercise following immobilization resulted in significant decreases compared to cage activity in range of motion, tendon stiffness, modulus, percent relaxation and several parameters from both a structurally based elastic model and a quasi-linear viscoelastic model. Therefore, we conclude that after a short period of immobilization, increased activity is detrimental to both tendon mechanical properties and shoulder joint mechanics, presumably due to increased scar production.

Introduction

Rotator cuff tears are a common clinical problem that can result in significant disability and often require surgical repair.[1] Unfortunately, re-tear rates following surgical repair of the torn tendon to bone insertion site are quite high, occurring in 20–90% of cases.[1–3] A variety of studies have investigated strategies to enhance the healing of the insertion site in hopes of reducing repair failure rates. These studies have focused on both biological interventions, such as the addition of growth factors, as well as mechanical factors such as the appropriate post-operative rehabilitation protocol.

Our group has previously investigated the role of post-operative activity on tendon to bone healing in a rat model. We found that post-operative immobilization resulted in superior tendon to bone healing properties compared to both cage activity and exercise.[4, 5] These properties

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also continued to improve with increasing time of immobilization, with positive changes in extracellular matrix expression seen as early as 2 weeks. Increased collagen organization at the insertion site was seen after 4 weeks of immobilization and superior mechanical properties were found at 8 and 16 weeks.[4, 5] Additionally, long periods of immediate post-operative exercise resulted in worse properties compared to immobilization and cage activity.[4] These results are consistent with a mouse fracture healing model in which loading immediately after injury was found to be detrimental, although delayed loading was beneficial.[6]

Although immobilization following rotator cuff repair surgery has gained popularity clinically in recent years[7], changes in joint mechanics and tendon properties following differing post-immobilization activity protocols have not yet been determined. Based partly on the delayed loading benefits seen in bone, it is the overall hypothesis of this research program that it is possible increased loading could be beneficial in this model after a period of immobilization to protect the healing insertion site. Therefore, the objective of this study was to determine the effect of activity level on insertion site mechanical properties and shoulder joint mechanics following supraspinatus injury, repair and a short period of immobilization. Our hypotheses were: 1) a long period of exercise after a short period of immobilization would cause detrimental changes in insertion site properties compared to cage activity and 2) differing post-immobilization activity levels would not result in changes in passive shoulder mechanics.

Materials and Methods

Animal Experiment

Twenty-two male Sprague-Dawley rats (Charles River, 400–450g) were used in this study approved by the Institutional Animal Care and Use Committee. All animals underwent a unilateral supraspinatus detachment and repair surgery, as previously described.[8] Briefly, incisions were made through the skin and superficial shoulder musculature before sharply detaching the entire supraspinatus tendon from its insertion on the humerus. Before repair, any remaining fibrocartilage at the insertion site was removed using a high-speed burr in order to allow for recreation of the insertion site. A drill hole was then made through the humerus and the tendon was grasped using a modified Mason-Allen technique. The suture was passed through the drill hole and the tendon was reapposed to its insertion and the incision was closed in a layered fashion.

Immediately following surgery, all rats were immobilized for 2 weeks in a manner similar to that described previously.[5, 9] Briefly, Webril (Tyco Healthcare) was placed around the injured arm and upper torso, forming a modified sling. This Webril sling was then covered in a layer of adhesive bandage (Vetrap, Penn Vet Supply). After the initial 2 weeks of immobilization, rats were separated into 2 groups based on their remobilization activity level: cage activity (CA, n=8) or exercise (EX, n=14). Animals assigned to the exercise group underwent controlled and gradual remobilization by moderate treadmill running. In the first week following the end of immobilization, the EX group was prescribed cage activity only. Following this initial week of cage activity, rats in the EX group ran on a treadmill for 5 days/week at a moderate speed of 10 m/min beginning with 7 minutes on the first day and gradually increasing over a 3 week period to 60 minutes/day. The animals then continued to run for 60 minutes/day, 5 days/week for the remaining 8 weeks of the study. The CA group received cage activity only for the same 12 week period.

Joint Mechanics

Passive range of motion was measured for all animals prior to surgery, immediately following the immobilization period, and at 12 weeks post repair, similar to that described.[9] At each time point, the animal was anesthetized and its arm placed in a rotating clamp at its neutral position

(90° of forward-flexion and 0° of abduction). A torque was then applied to the arm for three internal and external rotation loading and unloading cycles to a prescribed torque target. Range of motion was determined using data from all three cycles.

Mechanical Testing

After sacrifice, muscle-tendon-bone segments were dissected out for mechanical testing. The associated muscle was removed and fine dissection of the tendons was performed under a microscope. During this fine dissection, gross scar formed at the insertion site was removed by an experienced investigator in a consistent manner, while blinded to specimen group. Any scar tissue that was not well-formed enough to bear load was removed while any tissue that was observed to bear load was left intact. Four Verhoeff stain lines for optical strain measurements were then placed along the length of each tendon using 6-0 silk suture. The first stain line was placed at the insertion site (defined as the apposition of tendon into bone), the second 2mm proximally, the third 4mm proximally and the fourth and final stain line indicating grip placement was placed 8mm proximal to the insertion site. Cross-sectional area of the tendon from the insertion site to the third stain line was measured using a laser-based system.[10] For biomechanical testing, the humerus was embedded in a holding fixture using polymethylmethacrylate (PMMA) and the holding fixture was inserted into a specially designed testing fixture. The proximal end of the tendon was then held at the fourth stain line (8mm) in a screw clamp lined with fine grit sandpaper. The specimen was immersed in a 39° C PBS bath, preloaded to 0.1N, preconditioned for 10 cycles from 0.1N to 0.5N at a rate of 1%/sec, and held at 0.1N for 300sec. Immediately following the 300 second hold, a stress relaxation experiment was performed by elongating the specimen to a strain of 5% at a rate of 5%/sec (0.4 mm/sec) followed by a 600sec relaxation period. Following the 600 second relaxation period, specimens were returned to their initial preload displacement and held for 60 seconds. After the hold, a ramp to failure test was applied at a rate of 0.3%/sec. Peak and equilibrium load were determined from the stress relaxation test. Peak stress, equilibrium stress and percent relaxation were then calculated from these values. Using the applied stain lines, local tissue strain during the ramp to failure test was measured optically with a custom texture-tracking program (MATLAB). Elastic properties, such as stiffness and modulus, were calculated using a linear regression from either the visually determined linear region of the load-displacement curve (stiffness) or the stress-strain curve (modulus).

Structurally Based Elastic Model

In addition, load-displacement data were analyzed with a structurally based elastic model first developed by Lanir.[11] This function assumes that tendon is comprised of a population of linearly elastic fibers that un-crimp as the tendon is lengthened. After un-crimping, the fiber contributes to the force in a linear fashion, and the length at which the fiber un-crimps is referred to as the fiber's slack length. The tendon's non-linear force-length behavior, therefore, is the result of the non-linear distribution in fiber slack-lengths, which can be described by a cumulative probability distribution function (such as a Gaussian) described in the following:

$$p(L_0)=\frac{1}{\sigma\sqrt{2\pi}}\int_{-\infty}^{L_0} e^{\frac{-(t-\mu)^2}{\sigma}} \qquad (1)$$

Where p is the cumulative probability of a fiber being uncrimped, or recruited (between 0 and 100%), σ is the standard deviation of the fiber slack-lengths (mm), L_0 is the fiber slack-length (mm) and μ is the average fiber slack length (mm). A fiber is then uncrimped (recruited) once the tendon length has exceeded the fiber's slack-length according to:

$$F(x)=K_{avg}\sum_{i=1}^{N}(x-L_0^i)\cdot H(x-L_0^i) \tag{2}$$

where K_{avg} is the average fiber stiffness (N/mm), x is the tendon length (mm), L_0^i is the slack length of fiber i, and H is the heavy side step function (which is defined as 0 when $x< L_0^i$, and 1 when $x>=L_0^i$). This model has been used by Lanir and others to describe the non-linear elastic behavior of a variety of tissues. Generally speaking, disorganized tissues, such as skin, have a long flat toe-region, resulting in a large slack length mean and standard deviation; whereas, highly aligned tissue, such as ligament, have a relatively short sharp toe-region, resulting in a small slack length mean and standard deviation..12, [13] Thus, the more aligned or organized the tissue, the smaller the slack length standard deviation, Force displacement data from the ramp-to-failure portion of the test were fit to this structurally based model using the nonlinear least squares function in MATLAB to determine slack length distribution parameters (mean and standard deviation) and average fiber stiffness. Average fiber stiffness and linear region stiffness were compared using a paired t-test.

Quasilinear Viscoelastic Model

Lastly, data from the stress-relaxation portion of the test were analyzed using quasi-linear viscoelastic (QLV) theory. Quasi-linear viscoelastic theory separates the instantaneous non-linear elastic behavior from the time-dependent relaxation behavior according

$$F(t)=\int_0^t G(t-\tau)\frac{\partial F^e}{\partial \tau}d\tau \tag{3}$$

where F(t) is the tendon force, G(t) is the relaxation function, and dFe/dt is the time derivative of the elastic function. In a manner consistent with Lanir[11], we used the structurally based elastic function in our QLV model. Specifically, we slightly modified Lanir's formulation by using the reduced relaxation function originally proposed by Fung14, since it is more commonly used in tendon and ligament literature 15:

$$G(t)=\frac{1+C\,[E_1 t/\tau_2)-E_1(t/\tau_1)]}{1+C\,\ln(\tau_2/\tau_1)} \tag{4}$$

where G is the reduced relaxation function, t is time, C is the relaxation factor, E is the exponential integral function, and τ_1 and τ_2 are the short and long relaxation time constants, respectively. Load displacement data from the stress relaxation test were fit using the nonlinear least squares function in MATLAB to determine slack length distribution parameters (mean and standard deviation), average fiber stiffness, as well as the reduced relaxation function constants (C, τ_1 and τ_2).

Parameters were then compared between groups using two-tailed t-tests with significance at $p<0.05$ and trend at $0.05<p<0.01$.

Results

Joint Mechanics

At the end of the remobilization period, corresponding to 14 weeks post surgery, range of motion was significantly decreased by approximately 15% in animals assigned to the EX group when compared to the CA group (Figure 1A).

Mechanical Testing

Tendon cross-sectional area was increased by approximately 50% in the EX compared to the CA group (Figure 1B). The stress-relaxation experiment showed that percent relaxation significantly increased (Figure 1C) in the EX group compared to CA. Data from the ramp to failure test showed a decrease in tissue modulus in the EX group (Figure 1D) as well as a significant decrease in tissue linear region stiffness (Figure 2).

Structurally Based Elastic Model

For the ramp to failure data, the slack length mean (μ) and standard deviation (σ) were significantly increased in the EX group compared to CA (Figure 3). In addition, average fiber stiffness was significantly less for the EX compared to the CA group, as was the linear region stiffness. Lastly, average fiber stiffness was not significantly different (Figure 2) than the stiffness obtained from linear region of the load-displacement curve.

QLV Model

The slack length mean (μ) was not significantly different between groups, but the standard deviation (σ) was significantly increased in the EX compared to CA group (Figure 4). In addition, average fiber stiffness was larger (trend, $p=0.06$, Table 1) for the EX compared to the CA group. The relaxation factor was also larger (trend, $p=0.1$, Table 1) for the EX compared to the CA group, and there were no significant differences between the natural log of either the short or long time constants (Table 1).

Discussion

We hypothesized that after two weeks of immobilization, exercise would be detrimental to tendon properties compared to cage activity and that post-immobilization activity level would have no effect on shoulder joint mechanics. Our results support the first hypothesis, as tendon cross-sectional area, several mechanical properties and fiber slack length parameters were significantly worse in the group that exercised compared to cage-activity following the short period of immobilization. Our second hypothesis, that post-immobilization activity level would have no effect on shoulder joint mechanics, was contradicted by the finding that the total range of motion was less for animals that received 12 weeks of treadmill running instead of cage activity after the short immobilization period.

This phenomenon of detrimental effects due to early increased loading has also been seen in cyclic loading of healing bone in the mouse tibia.[6] However in this study, it was also shown that while loading immediately after fracture inhibited healing, delayed loading was beneficial. Similarly, previous studies in the rat rotator cuff have shown that increased activity level immediately after injury and repair was detrimental to tendon to bone healing.[4, 5] Therefore, it is possible that a potential benefit of increased activity following cuff repair could be realized after a period of immobilization to protect the early healing of the repair.

In the current study, we chose to evaluate the effect of increased post-immobilization activity after the shortest period of immobilization previously shown to result in beneficial changes to the healing insertion site. Specifically, we previously showed that 2 weeks of immobilization immediately following supraspinatus tendon injury and repair resulted in increased expression of extracellular proteins more like a normal insertion site when compared to both immediate post-operative exercise and cage activity.[5] In the current study, we found that after 2 weeks of immobilization, increased loading (exercise versus cage activity) resulted in increased tendon cross-sectional area and fiber slack length parameters as well as a decrease in mechanical properties. These results are consistent with a recent mouse tibia osteotomy healing study, which also found that increased loading produced more bone (as measured by bone mineral content) but decreased mechanical properties.[6] Although there are several differences between the mouse study and our own, they both indicate that increased loading too soon following injury can lead to increased matrix production, but inferior mechanical properties. We interpret these results to suggest that at 2 weeks following rotator cuff repair, the repaired insertion site is still at an ill-formed state, and therefore, increased loading serves only to produce more disorganized "scar" tissue. Although we have not measured tissue organization directly, results from the structural model indicate that increased loading increased slack-length variability, which is consistent with less organized tissue.13 Indeed, these differences were apparent for both the stress-relaxation test and the ramp to failure test, which both showed significantly less organization for the exercise group, despite the differences in pre-loads, displacement rates and maximum displacements between the two tests. Additionally, this interpretation also explains the in-vivo passive shoulder mechanical data in that increased scar formation would limit shoulder range of motion.9 It is possible that increased loading following a longer period of immobilization, after which the tissue at the repair site is well-organized, may produce more of the desired tendon tissue rather than scar, resulting in superior mechanical properties, and will be the subject of future studies. Finally, the application of the structurally based elastic model in order to determine stiffness and modulus during the ramp to failure test was found to agree quite closely with our established methods to calculate linear region stiffness and modulus from the load-displacement and stress-strain curves, respectively. These results further validate the use of this model with our data and provide confidence in our findings regarding tendon fiber slack length properties.

As with any study, there are several limitations which must be taken into account when interpreting the results of this study. First, we did not measure tissue organization directly. As a result, our interpretation that increased loading led to increased scar formation is based on indirect data (decreased stiffness, decreased structural model organization and decreased ROM) and gross observations during specimen preparation. While the structural model has not been validated in rat supraspinatus tendon specifically, several authors have previously shown in ligaments that the structural models estimates of fiber slack length correlate with the lengths at which fibers un-crimp during tensile loading and therefore describe tissue structure.[16, 17]

In conclusion, after a short period of immobilization, an increased level of activity is detrimental to both tendon mechanical properties and shoulder joint mechanics. Therefore, future studies will examine the effect of post-immobilization activity modification after longer immobilization which has been previously found to improve collagen organization or mechanical properties of the repaired tendon to bone insertion site.

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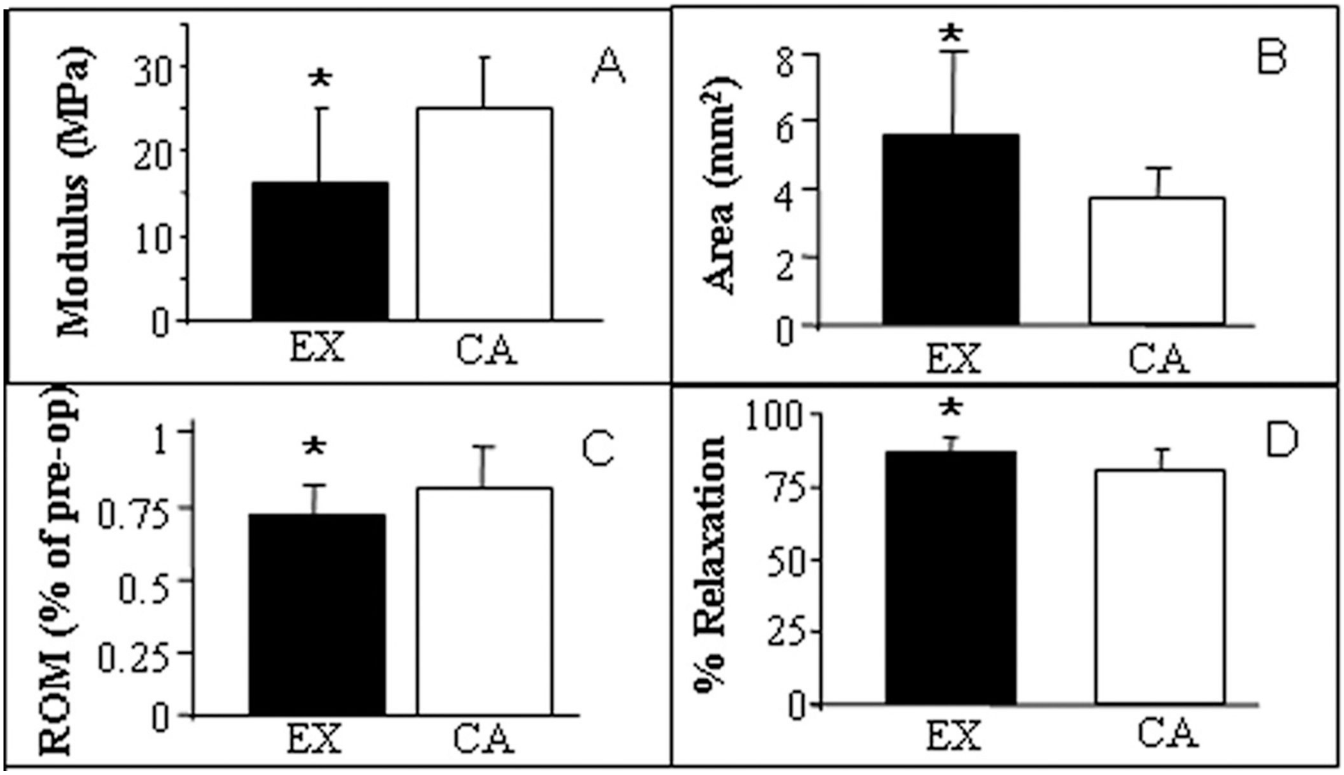

Figure 1.
Detrimental changes in tendon properties (A: modulus, B: area, C: total ROM, D: percent relaxation) seen with post-immobilization exercise compared to cage activity (*=p<0.05)

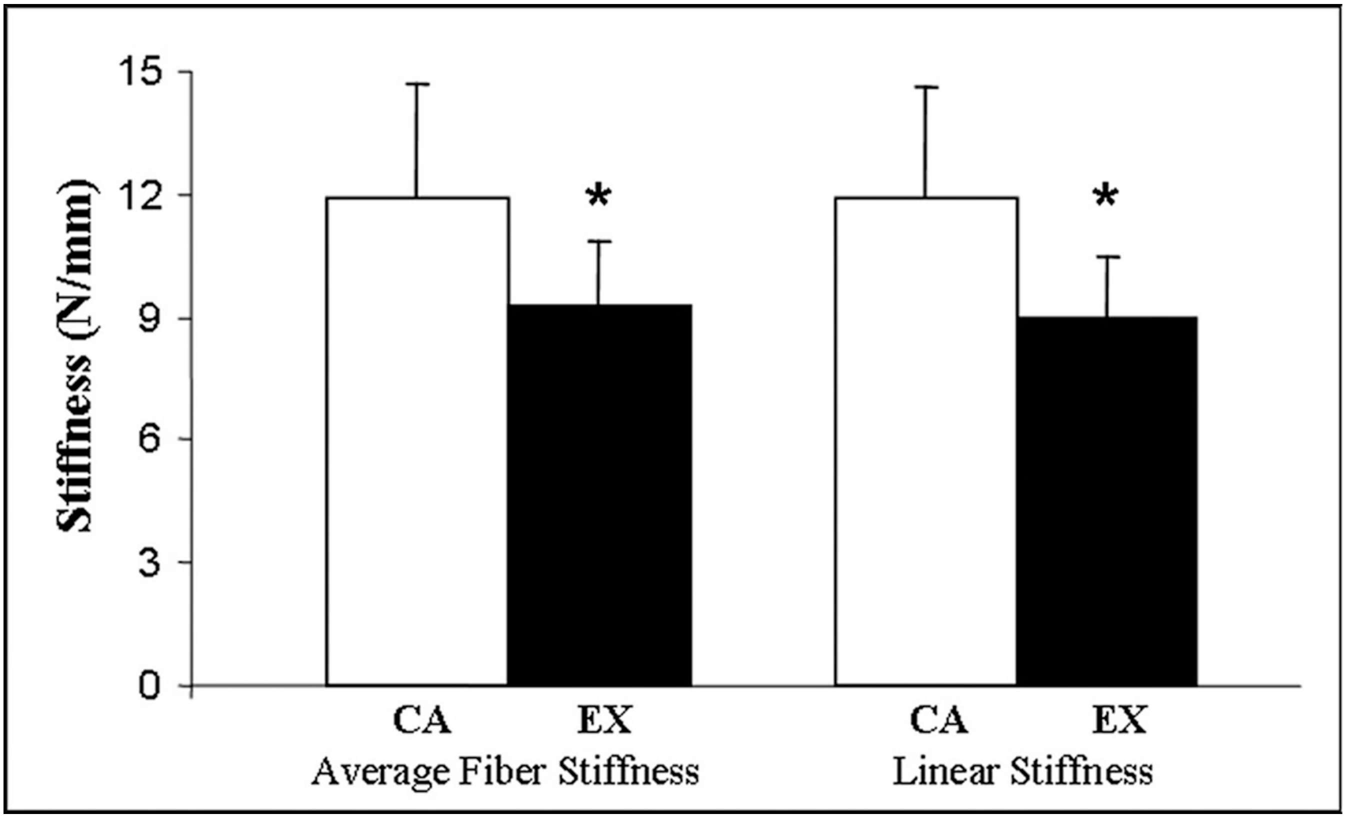

Figure 2.
Stiffness is decreased with EX compared to CA following immobilization (*=p<0.05). This is seen in both average fiber stiffness (as calculated from the model) and linear stiffness (as calculated from the slope of the load-displacement curve). In addition, the respective stiffness values within a group are not different.

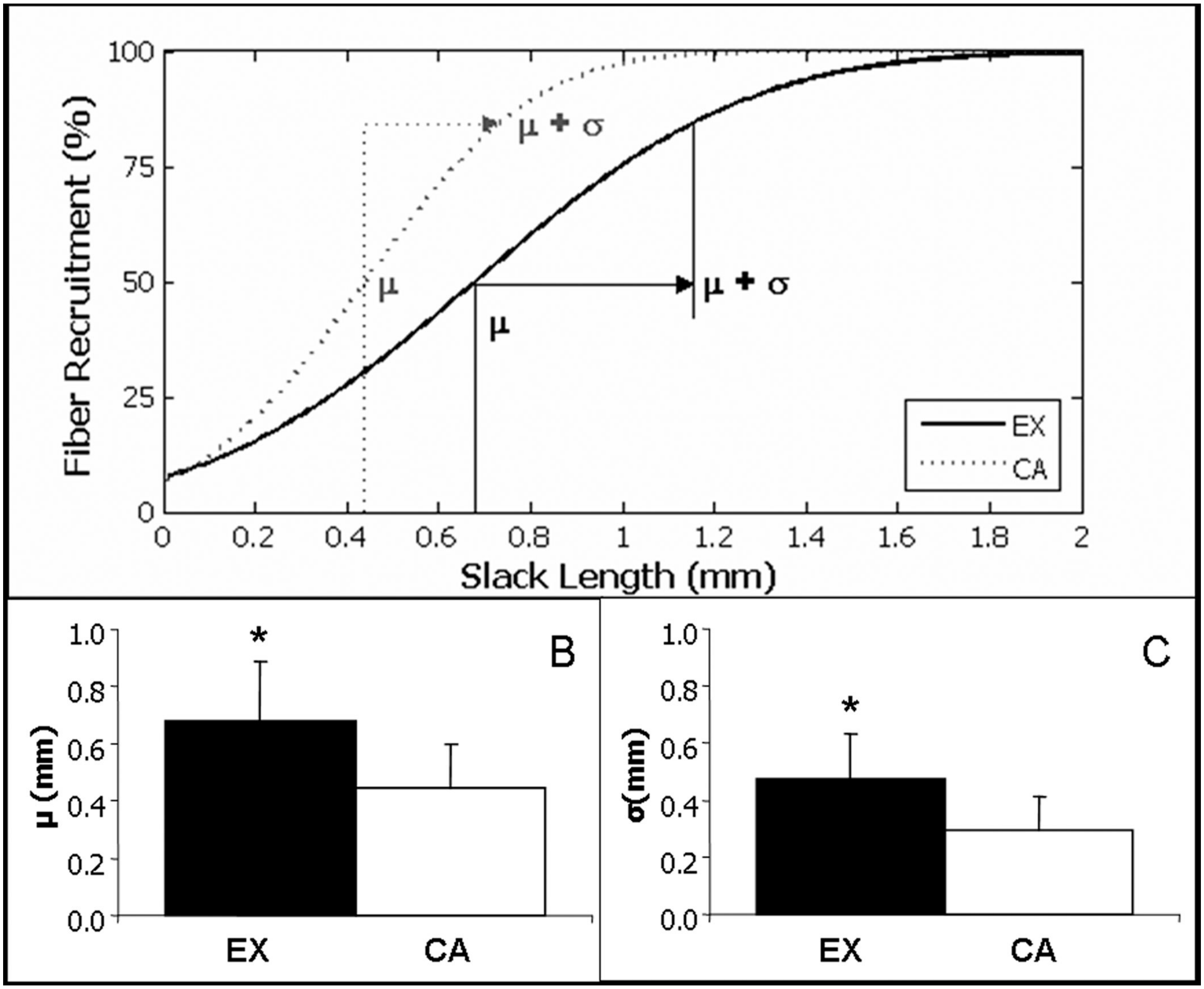

Figure 3.

Parameters determined from the structurally based elastic model [mean slack length, μ (A,B), and slack length standard deviation, σ (A,C)] are increased with EX compared to CA (*=p<0.05).

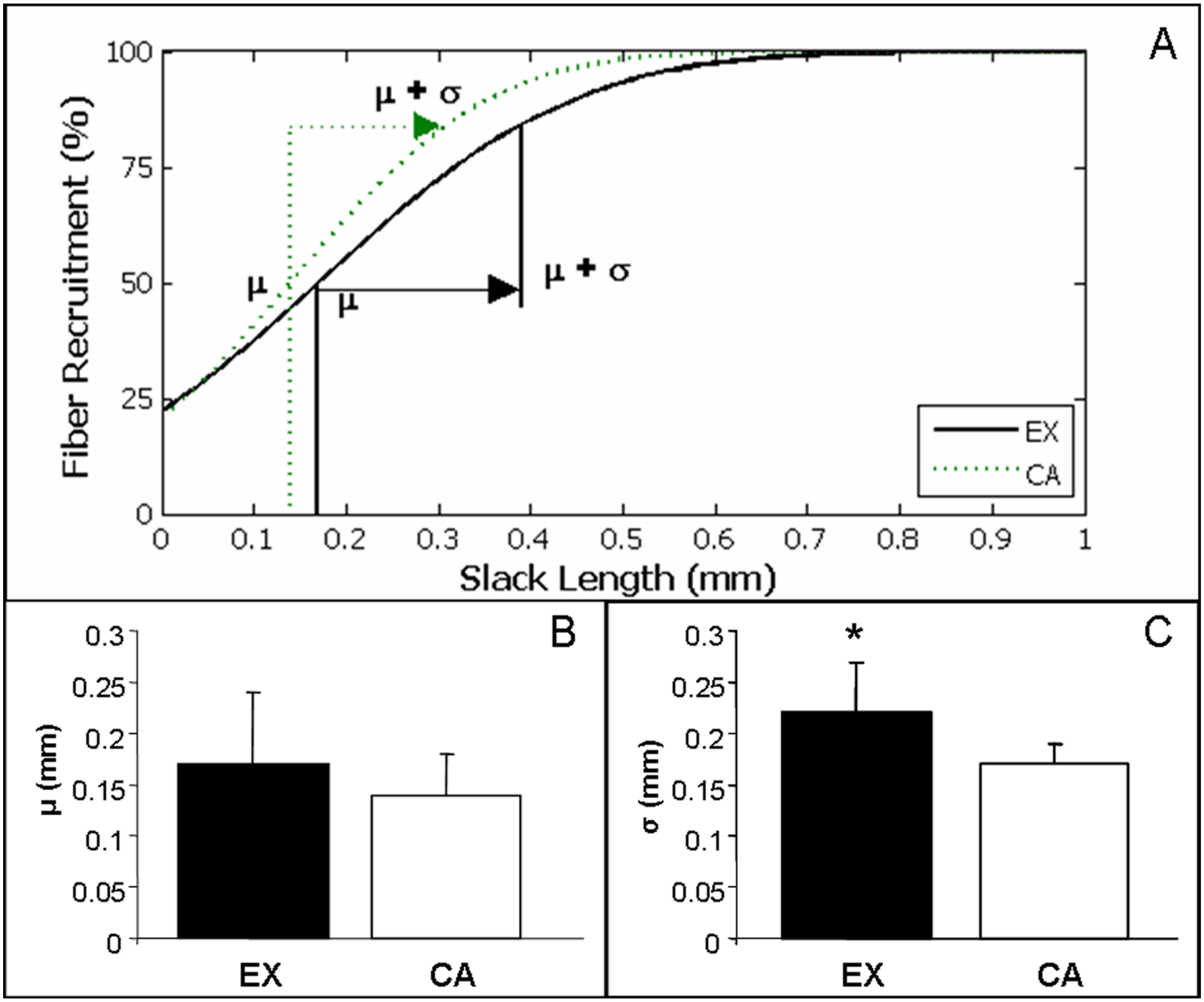

Figure 4.
During the stress-relaxation test, slack length standard deviation from the QLV model, σ (A,C), is increased with EX compared to CA but mean slack length, μ (A,B), is unchanged.

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Table 1

Average ± standard deviation quasi-linear viscoelastic parameters for the caged-activity (CA) and exercised (EX) groups (* = $p<0.05$, # = $p<0.1$). Slack length mean (μ), standard deviation (σ), elastic element stiffness (Kel), relaxation factor (C), the short time constant (T1) and long time constant (T2).

Group	μ (mm)	σ (mm)	Kel (N/mm)	C	ln(T1) (sec)	ln(T2) (sec)
CA	0.14 ± 0.04	0.17 ± 0.02	11.5 ± 3.6	0.9 ± 0.4	−1.3 ± 0.7	5.5 ± 0.5
EX	0.17 ± 0.07	0.22 ± 0.05*	8.3 ± 2.8#	1.3 ± 0.5#	−1.0 ± 0.3	5.7 ± 0.4