

THE ACCUMULATION OF DAMAGE UNDER CYCLIC LOADING IS NOT ALTERED FOLLOWING TRAUMATIC STRAIN IN THE RABBIT MCL

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INTRODUCTION

Ligaments of the knee are regularly subjected to repetitive loading *in vivo*. Typically, *ex vivo* mechanical studies of ligaments focus on strength or on repetitive loading protocols of short duration [e.g.: 1]. While recent studies of damage to ligament tissue have investigated damage at the cellular [2] and structural levels [2, 3], tests of damage accumulation with repetitive loading, that is fatigue, are lacking. Some authors have investigated the influence of fatigue loading on tendon tissue [e.g.: 4, 5], but to our knowledge, no study of the fatigue behaviour of ligament tissue has been conducted.

The aim of this study was to characterize the mechanical behaviour of the rabbit medial collateral ligament (MCL) subjected to fatigue loading with or without prior sub-failure strain. We hypothesized that ligament tissue accumulates damage with repetitive loading and that ligaments subjected to sub-failure strains will show greater evidence of damage and that damage will accumulate at a faster rate when compared to controls.

METHODS

Fourteen ligaments from eight skeletally mature, female NZW rabbits (average mass: 5.3 ± 0.9 kg) were utilized in this study approved by our institutional animal care committee. Animals were divided into two groups consisting of a control group ("Normal") and a group subjected to sub-failure elongation ("Stretched").

In vitro mechanical testing: Limbs were mounted and preconditioned at approximately 70° of flexion in custom-designed clamps in a servohydraulic testing machine (MTS Systems, Eden Prairie, MN, USA) as per our standard protocol [1]. Control ligaments were then buckled (-1 mm) and soaked in a 10% sucrose solution at 37°C for one hour. Ten per cent sucrose is approximately isotonic for normal adolescent rabbit MCLs [1]. Experimental ligaments were loaded cyclically 30 times to 28 MPa at 1 Hz to determine the pre-stretch stiffness. These ligaments were then stretched under stroke control to a displacement of 3.0 mm at 100mm/s. Ligaments were

returned to "ligament zero" and subjected to 30 more cycles (28MPa) to determine post-stretch stiffness. Finally, the ligaments were buckled and soaked for one hour in 10% sucrose. EDTA (~2 mM) and Gentamicin (~0.05 mg/mL) were added to the sucrose solution to inhibit proteolytic and bacterial activity respectively. Following equilibration in 10% sucrose solution for one hour, all ligaments were loaded repetitively between "ligament zero" and 28 MPa (1 Hz, 10% sucrose, 37°C). Ligaments in the control group were cycled to failure or until 259200 cycles, whichever came first. The stretched ligaments were loaded for 24 hours (86400 cycles) and then failed at an extension rate of 20 mm/min. **Outcome measures:** The accumulation of damage with loading was monitored by determining the stiffness of each ligament on the first, 30th, 1800th, 18000th, 36000th, 54000th, 72000th and 86400th loading cycles. Complete failure or a reduction in residual strength were additional indices of damage with residual strength constituting the failure load (ramp-to-failure at 20 mm/min) of an experimental ligament. Data were analyzed using Student's t-tests (between groups) or a General Linear Model with Bonferroni's post-hoc test (within groups).

RESULTS

In the control group, seven ligaments were cycled to failure or to 259200 cycles. One ligament failed in less than 24 hours of loading (31031 cycles). Of the remaining six, three lasted 259200 cycles before loading was discontinued. Stiffness increased significantly between the first cycle and 1800th, 18000th, 36000th and 54000th cycles ($p < 0.04$) (Figure 1). Stiffness was significantly decreased for the 86400th cycle when compared with the 1800th to 54000th cycles ($p < 0.004$). For the intervals examined, stiffness was greatest during the 18000th cycle at $121.6 \pm 17.7\text{N/mm}$. By the 36000th cycle of loading, 4 out of 7 samples showed a reduction in stiffness. The mean reduction in stiffness after 24 hours of loading was $7.0 \pm 5.1\%$ ($n = 6$). Ligament length increased significantly between the 1st cycle and 1800th to 86400th cycles ($p < 0.0001$) (Figure 2). No significant differences in length between intervals were detected after the 1800th cycle.

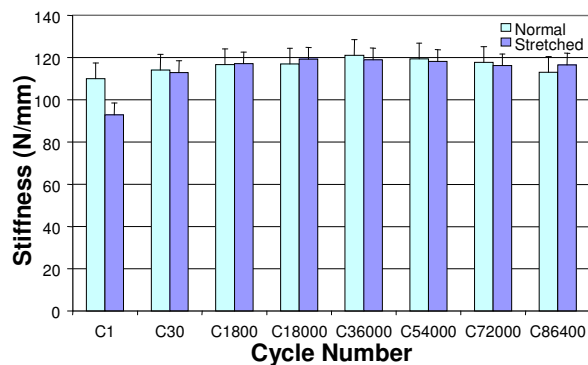


Figure 1. MCL stiffness during cyclic loading. C1 = Cycle 1.

One ligament in the stretched group failed before 24 hours of loading (69 cycles) and was therefore excluded from analysis. The reduction in stiffness following sub-failure stretch is shown in Table I with a mean of $6.1 \pm 1.9\%$ ($p = 0.0001$). Stiffness was significantly greater than the 1st cycle for all subsequent intervals ($p < 0.0001$) (Figure 1). No significant change in stiffness was noted between intervals after the 30th cycle. Stiffness was greatest during the 18000th cycle at 119.2 ± 13.0 N/mm. By the 36000th cycle of loading, 4 out of 6 samples showed a reduction in stiffness. Unlike the control group, stiffness was not significantly decreased for the 86400th cycle when compared with the 1800th to 54000th cycles. Ligament length increased significantly between the 1st cycle and 1800th to 86400th cycles ($p < 0.0001$) (Figure 2). No significant differences in length between intervals were detected after the 1800th cycle. The mean failure load for this group was 319.2 ± 64.4 N.

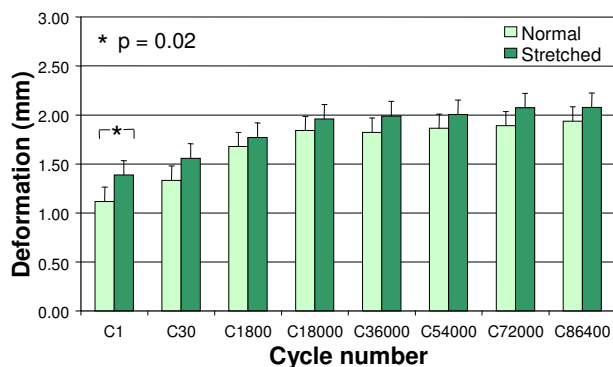


Figure 2. MCL Deformation during cyclic loading.

There were no significant differences between the stiffnesses of the control and experimental ligaments. A significant difference in elongation was only noted for the first cycle of loading ($p = 0.02$). Following the one hour soak in 10% sucrose solution, a reduction in stiffness was noted in the 'Stretched' ($p < 0.005$) group when compared with the mean stiffness prior to soaking. This reduction was no longer evident after 1800 cycles of loading ($p > 0.49$).

DISCUSSION

The results demonstrate that rabbit MCLs accumulate damage under repetitive loading at a moderate stress level (28 MPa) – about 30% of ultimate strength. It is important to note that damage, as indicated by

Table I. MCL stiffness: pre and post-ramp.

Sample	Cyclic Load (N)	Pre-Ramp Stiffness (N/mm)	Post-Ramp Stiffness (N/mm)	% Reduction in Stiffness
5b	66.4	104.7	97.3	7.1%
7a	89.0	127.1	119.0	6.3%
7b	86.9	109.7	100.0	8.8%
9b	79.5	124.8	119.3	4.5%
10a	80.9	141.1	136.3	3.4%
10b	85.7	139.3	130.7	6.2%
Mean	81.4	124.4	117.1	6.1%
SD	8.2	14.9	15.8	1.9%

reductions in stiffness, was not evident until after approximately 10 hours of loading (36000 cycles). The contribution of fibre recruitment, water exudation or other potential mechanisms will have to be investigated to determine their relative contribution to increasing MCL stiffness and/or subsequent damage.

The MCLs subjected to rapid sub-failure strains did not accumulate damage faster than control ligaments. This may be because the overall reductions in stiffness (mean 6.1%) in the stretched ligaments were small following the application of a sub-failure stretch. The onset of reduction in stiffness occurred at the same time point as for controls. The net reduction in stiffness for both groups (7.0% vs. 8.2%) was similar. The mechanism by which the 'stretched' ligaments were able to resist further accumulation of damage needs to be elucidated. Additionally, ligaments subjected to sub-failure strain and 24 hours of cycling were not weaker on average (319.2 N) than values previously determined in our lab or cited in the literature [e.g.: 3].

Previous authors have demonstrated the effect of altering tissue water content on the viscoelastic behaviour of the rabbit MCL [1]. This study also demonstrated that, for the stretched tissues, soaking in 10% sucrose for one hour reduced the initial stiffness of these tissues. However, within half an hour of cycling, this effect was removed, suggesting that tissues bathed in solution likely exude water while undergoing cycling.

This study has demonstrated that normal rabbit medial collateral ligaments can withstand over 250,000 repetitive loading cycles in an *ex vivo* environment with minimal damage. This suggests that MCLs may be structurally optimized to resist damage *in vivo* under moderate repetitive loads.

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