INTRODUCTION: Progressive damage to the “secondary restraints” in the ligament-injured knee has long been recognized [e.g. 1]. Rupture of the Anterior Cruciate Ligament is associated with a high incidence of secondary damage to the remaining intact ligaments. Some potential mechanisms of this secondary damage have been investigated [e.g. 2], but the exact process remains unclear and moreover prevention strategies for this secondary damage are lacking. One possible explanation is that ligament swelling (associated with joint inflammation) may predispose ligaments to load-induced insult. Recognizing that intact ligaments may be subjected to higher in vivo stresses following ACL rupture, we wanted to systematically study the influence of applying repetitive loads on the accumulation of fatigue damage in an in vitro model and simultaneously determine the role elevated tissue water content might play in the accumulation of damage. We hypothesized that the greatest reduction in ultimate tensile strength (evidence of fatigue damage) would be observed in the samples subjected to the greatest number of load cycles and those with the highest water content. As ligament creep has been shown to be correlated with tissue water content [3], we also hypothesized that elevated ligament water content could lead to further damage accumulation potentially via a creep-mediated mechanism.

METHODS: Thirty-five medial collateral ligaments from skeletally mature, female NZW rabbits were utilized in this study approved by our animal care committee. Water content was manipulated by testing ligaments in either a hypotonic (0.1%) or an isotonic (10%) sucrose solution [MCL water contents: 75.7±6.7% and 65.6±0.4% respectively]. To separate time-dependent creep strain effects from fatigue effects (cycle number dependent) ligaments were tested at one of two frequencies (1 Hz and 0.1 Hz) and for one of two durations (2.4 hours or 24 hours). Control samples were subjected to 30 cycles of loading to establish baseline values for cyclic displacement and were then left to soak in solution for 24 hours. Loading duration for the four groups were as follows: controls – 30 cycles at 1 Hz (30s), “fast” – 8640 cycles at 1 Hz (2.4 h), “slow” – 8640 cycles at 0.1 Hz (24 h) and “long” – 86400 cycles at 1 Hz (24 h). In the above fashion, the influence of cycle number, test duration and water content on damage accumulation could be investigated. In vitro mechanical testing: Limbs were mounted and preconditioned at ~70° of flexion in custom-designed clamps in a servohydraulic testing machine (MTS Systems, USA). Ligament were ‘made slack’ (displacement to -1 mm) and soaked in sucrose solution (37°C, with protease inhibitors) for one hour. Following equilibration, experimental ligaments were loaded repetitively between 0.1N and a load corresponding with 28 MPa (~20% UTS). After 24 hours, all samples were allowed to recover at 0.1 N for one hour. Ligaments were then cycled 30 times to assess mechanical behaviour post-recovery and then ramped to failure at a rate of 20 mm/min (Fig. 1).

RESULTS: The ultimate tensile strength of ligaments cycled for 24 hours in hypotonic solution (both 0.1 Hz and 1 Hz) were significantly less (p < 0.02) than those measured for ligaments tested in isotonic solution (Fig 2). Ligaments in the control groups showed no sign of damage as they did not demonstrate reductions in UTS. When tested in hypotonic solution, ligaments from the loaded test groups lengthened more than those cycled in isotonic solution (data not shown). These differences were significant for all time points for the group loaded at 0.1 Hz. Interestingly, within test groups (i.e. isotonic and hypotonic), final lengths were not significantly different for ligaments loaded for 2.4 hours vs. 24 hours. Both qualitative and quantitative mechanical differences were apparent in loaded ligaments after a period of loading and recovery: minimum length values (l_mn) increased at a faster rate, maximum displacements were greater and the shape of the second derivative plot changed markedly within 30 load cycles (Figure 3).

Our first hypothesis could explain some of the observed findings; however test duration appeared to be more important than the absolute number of load cycles with respect to damage accumulation. The second hypothesis was consistent with the data, but creep alone could not account for all of our observations. For example, final displacements were not different between samples loaded for either 2.4 or 24 hours, but their UTSs were different. Taking all our findings into account, it appears that fatigue and creep processes act in concert to weaken the tissue during loading and that tissue swelling promotes these effects.

REFERENCES:

A POTENTIAL MECHANISM FOR DAMAGE TO SECONDARY RESTRAINTS IN THE ACL DEFICIENT KNEE

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Figure 1. Schematic of Loading Protocol

Figure 2. UTS values.

Figure 3. Force-displacement plots with superimposed plots of d²F/dl². Arrows denote position of l_mn. Black and grey lines depict the first and 30th cycle of load-post-recovery. Cyan and blue lines depict plots 30 cycles apart with an equivalent value for l_mn as the 1st cycle post-recovery. This sample was cycled in hypotonic solution for 24h at 1 Hz.