INTRODUCTION

Ligament water content may be altered following injury or surgery. The aim of this experiment was to monitor the mechanical behaviour of the medial collateral ligament (MCL) in response to hypo- or hypertonic test environments \textit{in vitro}. We hypothesized that normal and healing MCLs would not only creep more in a hypotonic environment than in a hypertonic one, but would also recover more under hypotonic conditions.

METHODS

Thirteen skeletally mature, female NZW rabbits (average mass: 5.3 ± 0.5 kg) were utilized in this study approved by our animal care committee. Experimental animals underwent bilateral surgical transection of the MCL at the joint-line. As part of a parallel study, one joint had the anterior cruciate ligament (ACL) transected four weeks following MCL transection while the contralateral limb received a capsulotomy (ACL-intact). Data from the ACL-intact group are shown here. At six weeks, the animals were sacrificed, and the hindlimbs were rapidly dissected and prepared for mechanical testing. Five joints underwent mechanical testing while immersed in Dulbecco's modified Eagle's medium (DMEM); limbs from five other experimental animals were tested in 25% Sucrose solution. Age-matched, uninjured animals served as controls (n = 3). \textit{In vitro} mechanical testing: Limbs were mounted and preconditioned at 70° of flexion in custom-designed clamps in a servohydraulic testing machine (MTS Systems, Eden Prairie, MN, USA) as described previously [Zec \textit{et al.}, 2002]. After preconditioning and isolation of the MCL, a plastic bag affixed to the lower clamp was pulled up over the joint and the test solution (~23°C) poured into the bag. The MCL was then cyclically loaded between 0.1N and 30N for one hour (1 Hz). Ligaments were allowed to recover at zero load until the original length was achieved (data for the 1st hour shown). Outcome measures: Cyclic creep strain was defined as the strain at the peak of the 3600th cycle minus the strain at the peak of the first cycle. Unrecovered creep strain was defined as the residual strain after one hour of unloading at 0.1N. Unpaired Student’s t-tests were used for data analysis.

RESULTS

Water contents were higher for all MCLs tested in DMEM when compared to those tested in 25% Sucrose (normals: ↑10.7% (p < 0.06); healing: ↑20.1% (p < 0.001)). CREEP: Normal ligaments cycled in DMEM did not creep more than those cycled in 25% Sucrose (p > 0.9). Healing MCLs crept more than normals and crept more in DMEM than in 25% Sucrose (Figure 1). RECOVERY: When expressed as a percentage of total strain, normal ligaments tested in 25% Sucrose had proportionally higher values of unrecovered creep strain than those tested in DMEM (p < 0.006). Likewise, healing MCLs tested in sucrose also demonstrated a higher proportion of unrecovered strain when compared with MCLs soaked in DMEM (p < 0.001) (Figure 2).

DISCUSSION

Altering ligament water content has previously been shown to affect the creep behaviour of normal MCLs [Thornton \textit{et al.}, 2001] and healing MCLs creep more than uninjured controls cycled at the same load [Zec \textit{et al.}, 2002]. We have demonstrated for the first time that healing ligaments soaked in hypotonic solution (DMEM) creep more than those cycled in hypertonic solution (25% Sucrose). Interestingly, normal ligaments cycled in DMEM did not creep more than those cycled in 25% Sucrose. Since ligaments in this study were not pre-soaked in these solutions prior to cycling, these findings suggest that normal ligaments imbibe little, if any, water while undergoing cyclic loading. The differences in the water contents of the two normal groups may be attributed to the recovery portion of the test protocol. All MCLs demonstrated a higher proportion of unrecovered strain while soaking in 25% Sucrose. This implies that recovery rate is dependent upon tissue water content.

SUMMARY

Altering ligament water content has been shown to alter tissue creep and recovery behaviour. For normal MCLs, the tissue’s initial water content may be of greater influence on cyclic creep behaviour than the solution the tissue is cycled in. Healing ligaments, on the other hand, have been shown to be sensitive to the tonicity of the test solution, even without a period of equilibration prior to cycling.

REFERENCES