INTRODUCTION: Recent studies have demonstrated that there was an elevated number of myofibroblasts and an increase in the expression of α-smooth muscle actin (α-SMA), a myofibroblast marker, within joint capsules obtained from humans and rabbits with post-traumatic elbow joint contractures. The chronic stages of post-traumatic joint contraction have been investigated, but the acute stages and growth factor levels have yet to be analyzed. The objective of this study was to evaluate the mRNA levels of α-SMA, TGF-β1, and the ED-A domain of fibronectin (myofibroblast upregulators) in the posterior capsule during the early stages of post-traumatic joint contraction. Our hypotheses are (1) the mRNA expression of α-SMA, TGF-β1, and the ED-A domain of fibronectin would be increased in the posterior capsule of post-traumatic joint contractures during the early stages compared to the contralateral knee joint capsules and normal knee joint capsules of unoperated rabbits, (2) the immobilization time would have an effect on the expression of these molecules, and (3) myofibroblast numbers will be elevated in contracture tissue compared to control tissue during early immobilization.

METHODS: Institutional animal care committee approval was obtained. Post-traumatic joint contractures were stimulated in the right knees of four skeletally mature (12-18 months old, 5.4 ± 0.6kg) New Zealand White female rabbits by creating an intra-articular fracture and immobilizing the knee with a Kirschner wire (K-wire). This model was previously used in our laboratory. The rabbits were equally divided into four groups based on the time of immobilization: 0 weeks, 2 weeks, 4 weeks, or 6 weeks. Contralateral limbs served as unoperated controls. Normal knee joint capsules (n=6) were obtained from three age and gender matched rabbits. Hind limbs were removed and posterior joint capsules were harvested and immediately frozen for evaluation. Semi-quantitative RT-PCR was used to evaluate mRNA levels in all four groups, employing the TRIspin method. Rabbit specific primers for α-SMA, TGF-β1, and the ED-A domain of fibronectin were analyzed. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize the values of cDNA. Primer sets were optimized for PCR cycles and linear amplification curves were plotted. The approximate optical densities were analyzed. Myofibroblasts were counted in the 4-weeks immobilization group. An immunohistochemical procedure was employed using a double labeling technique: monoclonal antibodies to α-SMA and affinity purified antibodies to laminin. DAPI was then applied to label nuclei. This technique has been previously used in our laboratories. Statistical comparisons between contracture and control limbs were made with a paired t-test. An analysis of variance (ANOVA) with Bonferroni Tukey tests was used to evaluate the changes over time for the mRNA values. The normal knee joint capsules were included as one group in the ANOVA. The data are presented as mean ± standard deviation where p-values are significant if p ≤ 0.05.

RESULTS: All K-wires remained intact. Upon observation, there was a significant increase in α-SMA mRNA expression in the posterior joint capsule of contracture knees when compared to contralateral control knees in all four groups. It was at 0- and 2-weeks immobilization that there was a significant increase in expression in contracture knees compared to the normal knees. TGF-β1 mRNA expression was significantly elevated in contracture knees compared to control knees in all four groups, (Figure 1) and at 6-weeks there was a significant increase in expression in contracture knees compared to normal knees. The mRNA levels for the ED-A domain of fibronectin were significantly increased in the contracture group compared to the control group only at 0-weeks. For the remaining 6 weeks, there was no difference between contracture knees and control normal knees for the ED-A domain of fibronectin. At 4-weeks of immobilization, myofibroblasts were present in both control and contracture tissue (Table 1). Absolute myofibroblast numbers and percentage of myofibroblasts were significantly increased in contracture tissue compared to control tissue. There was no difference observed between total cells obtained from contracture knees and control knees.

DISCUSSION: The results presented reveal that a significant increase was observed in the mRNA expression of α-SMA, TGF-β1, and the ED-A domain of fibronectin (at 0-weeks) in contracture knees compared to control knees. The effect of immobilization time on the changes in the expression of these molecules was subtle; however, it was immediately upon injury that the mRNA levels of these molecules were up regulated. It was determined that myofibroblast numbers and the percentage of myofibroblasts were significantly elevated in contracture tissue when compared to control tissue. The cellular metabolism of the joint capsule genes were not all up-regulated in the contracture knees as other molecules (TIMPs) are down-regulated (data not shown).

These results are consistent with each other. TGF-β1 is a growth factor which promotes and regulates the expression of α-SMA and myofibroblast differentiation, thus, the elevated α-SMA and myofibroblast numbers are consistent with the elevated TGF-β1 shown in the study. The increase in expression of the ED-A domain of fibronectin is also significant because its interaction with TGF-β1 promotes the increase in α-SMA and myofibroblasts. These findings support previous studies conducted during the chronic stages of contracture where there was an increase in α-SMA expression and myofibroblasts in the contracture group compared to control groups. Interestingly, changes within molecular expression and cellular proliferation occur early on during the contracture process. The reasons for this immediate upregulation in molecular expression and cellular proliferation and differentiation are currently unknown. To our knowledge there are few studies which have investigated the early stages of joint injury and immobilization within joint contracture, the implications involved, and the possible changes to various growth factors. To have an understanding of the early process is imperative to fully comprehend the role of the growth factors and cells involved.

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REFERENCES: