Introduction: Tendon and ligament injuries heal reparationly by forming a less organized and mechanically inferior scar, whose properties improve over time but do not return to normal levels. In vivo studies have shown that injured tissues treated with exogenous cytokines exhibit improved mechanical properties when compared to untreated injured tissues [1,2]. However, these studies often report conflicting results due to the many factors that must be considered, such as how much, when, in what order, and in what combination these cytokines should be added. The availability of transgenic mice with cytokine knockouts allows for a more precise and targeted approach to assess tendon healing. Therefore, the objective of this study is to utilize interleukin-4 (IL4) and interleukin-6 (IL6) knockout mice in order to investigate their roles in tendon healing. IL4 is an anti-inflammatory cytokine that has been shown to stimulate fibroblasts, while IL6 is a pro-inflammatory cytokine that has been implicated to inhibit fibroblasts. With the absence of IL4 or IL6 during tendon healing, we hypothesize that the collagen organization and material properties of injured IL6 knockout mice will be more organized and greater than those of injured control mice and injured IL4 knockout mice.

Methods: 48 C57BL/6 control (CTL), 45 interleukin-4 knockout (IL4), and 46 interleukin-6 knockout (IL6) mice were used (IACUC approved study). From each group at 10 weeks of age, 16 mice were sacrificed uninjured and 9-11 mice underwent surgical injury. Using a 0.75 mm diameter punch (60% of tendon width), a full thickness transection was created in the middle of the left patellar tendon with the right tendon undergoing a sham surgery. All mice were allowed cage activity until sacrifice at 3, 6, or 12 weeks post injury for organizational or biomechanical assays.

For the organizational assay, tendons were processed with standard techniques (H&E stain) and viewed under polarized light. Images were taken at 5° increments and digitally analyzed using an automated system [3]. Collagen fiber distributions were compared using a Chi-Squared method to test goodness of fit and angular deviations of the collagen orientations (a measure of the distribution spread) were computed. For the biomechanical assay, dumbbell shaped patellar tendon specimens were prepared. The width and thickness of each tendon were measured to calculate cross-sectional area [4]. To determine mechanical properties, each tendon specimen underwent the following protocol in a 37°C saline bath – preconditioning, stress relaxation (ramp to 5% strain at 25%/s followed by 10 min relaxation), and test to failure (constant rate of 0.1%/s). Local tissue strain was measured optically [5]. For both assays, comparisons across groups were made with a one-way ANOVA followed by Fishers test with statistical significance set at p<0.05.

Results: Consistent with our hypothesis, the collagen fiber distribution for the uninjured IL4 group was statistically less organized than the uninjured CTL group (Figure 1). Fiber distributions were also significantly different between CTL, IL4, and IL6 at 12 weeks post injury. There was also a trend towards a higher angular deviation (less organized) for the uninjured IL4 group compared to both CTL and IL6 (Figure 2). As expected, the modulus and peak/equilibrium stress ratio for the uninjured IL6 group were significantly greater than that of both the CTL and IL4 groups (Figure 3). Unexpectedly, the maximum stress and modulus for the CTL and IL4 groups were significantly greater than that of the IL6 group at the 3 week post injury time point (Figure 3). There were no other differences in the other organizational or biomechanical properties.

Discussion: The objective of this study was to investigate the role of IL4 and IL6 in tendon healing through use of knockout mice to quantify collagen organization and mechanical properties in healing tendons. The lack of IL6 during tendon healing was expected to increase fibroblast activity and decrease inflammation, leading to the production of more collagen that is also more organized. The lack of IL4 during healing was expected to have the opposite effect – less collagen that is less organized. The uninjured data set supports these hypotheses. Not only was the IL6 group more organized than the IL4 group, but the mechanical properties were significantly higher as well. These findings were expected to continue or even be magnified post injury. However, the expected differences in an injured environment were actually found to be significantly decreased at 3 weeks post injury.

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Figure 1. Collagen fiber distribution for uninjured CTL, IL4, and IL6

Figure 2. Angular deviation (mean ± standard deviation)

Figure 3. Biomechanical properties (mean ± standard deviation)