The correlation analysis showed that the stiffness of the female ACL is almost of fibrils per unit area are lower in females’ ACLs than in males. This sex difference was significant. In the male population, the modulus of elasticity and stiffness were not significantly correlated to the corresponding ultrastructural measurements, including the number of fibers per unit area and percent area occupied by fibrils.

Materials and Methods: Six male and six female ACLs were randomly chosen from a pool of ten male and ten female ACLs that had been tested for their structural and mechanical properties. The samples were selected for TEM analysis immediately after tensile testing. The anteromedial bundle (AMB) and the posterior bundle (PLB) of both sections of the torn ACL were separated after tensile testing. Core sections (about 1 mm diameter) of both ABM and PLB of the ACL were isolated. The sections were divided into proximal, middle and distal regions. Tissue specimens for TEM analysis were obtained from each region. Based on the collected images, the number of fibrils per unit area (fibril concentration), the average fibril diameter, and the percent area occupied by fibrils (fibril area fraction) were calculated. Overall, a minimum of eighteen images from each ACL were analyzed (nine per bundle). Student t-tests were applied to determine any sex-based differences in the ultrastructural parameters of the ACL. Correlation tests were performed to assess the extent of linear relationship between ultrastructural and material (structural and mechanical) properties.

Results: Inspection of TEM images revealed possible sex differences in the ultrastructural characteristics of the ACL. Several of these characteristics were therefore carefully quantified and compared, including the means and standard deviations of the ultrastructural measurements for both female and male population as well as the p-value determined by t-tests. The number of fibrils per unit area ranged from 124.8/μm² to 165.8/μm² in females and from 144.1/μm² to 224.4/μm² in males. The average fibril diameter ranged from 53.4 nm to 64.3 nm in females and 46.6 nm to 60.7 nm in males. Finally, the fibril area fraction (% area of the micrograph occupied by fibrils) ranged from 34.0% to 47.9% in females and 42.8% to 47.6% in males.

The results of two sample t-tests showed that the mean number of fibrils per unit area was smaller for females (p = 0.03; Table 1). However, the average fiber diameter, number of fibers per unit area, and percent area occupied by fibrils did not differ significantly between the sexes. The p-value for this test was also 0.05. The Pearson product moment correlation, r, which reflects the extent of linear relationship between variables, was calculated for ultrastructural measurements versus linear elastic properties. For the female population, the ACL linear stiffness and modulus of elasticity were strongly and significantly correlated to fibril density (r = 0.96 and 0.97 respectively). Furthermore, the failure load was highly correlated to fibril area fraction (r = 0.77) but the correlation was borderline significant. In the male population, the modulus of elasticity and stiffness were not correlated to fibril density at all (r of 0.46 and 0.16). However, stiffness showed a strong correlation with fibril area fraction (r = 0.73) but not statistically significant. Finally, in the male population, the failure load and failure strength were strongly and significantly correlated to fibril area fraction (r of 0.96). In females, only 60% of variability in stiffness was explained by fibril area fraction. Because it has already been established that male ACLs have higher failure load and strength, it is not clear whether fibril area fraction is indeed a good indicator of the failure load in general. Interestingly, average fiber diameter was not a significant predictor of any of the properties in the ACL in either sex.

While these results establish a link between sex differences in mechanical properties and ultrastructure of the ACL, these processes that give rise to these sex differences are not fully understood. Our previous studies of gene expression in the human ACL (2) identified sex differences that may affect both synthesis of new collagen, as well as production of matrix metalloproteinases that mediate the degradation arm of collagen fibril turnover. These biochemical processes are likely to affect fibril diameter and area fraction, but precisely how they might do so, and how they are regulated or influenced by sex remain completely unknown.


Acknowledgements: The authors gratefully acknowledge the support of TTUHSC, TTUCOE, and the NIH (AR049767). Table 1. The ultrastructural measurements of the Human ACL based on donor sex (Mean ± SD).