

Differences in Development of Cartilage Layer at Various Tendon and Ligament Insertions

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INTRODUCTION:

Direct-type attachments, such as Achilles tendon (AT) enthesis, quadriceps tendon (QT) insertion, patellar tendon (PT) insertion, and anterior cruciate ligament (ACL) insertion, involve four transitional tissue layers: ligaments or tendons, two fibrocartilage layers (unmineralized and mineralized), and bone. The varying degree of stiffness in these layers reduces the stress concentration at the insertion site. Glycosaminoglycans (GAGs) in the fibrocartilage layers contribute to tissue elasticity, which confers resistance to tensile, shear, and compressive stresses, and thus, they are essential for load transmission.

Differences in the development of cartilage layers in AT, QT, PT, and ACL insertions are unclear. Moreover, because the mechanical environments for the AT, QT, PT, and ACL insertions differ, the development of the AT, QT, PT, and ACL insertions may differ.

Therefore, using quantitative morphometric evaluations, this study investigated the differences in the development of fibrocartilage layers in AT, QT, PT, and ACL insertions in rabbits. We hypothesize that the development of fibrocartilage layers in AT, QT, PT, and ACL insertions would differ when comparing the AT, QT, PT, and ACL insertions in rabbits.

METHODS:

Forty-eight male Japanese white rabbits were used in this study. Six were euthanized at different stages (days 1, 2, 4, 6, 8, 12, and 24 weeks). Rabbits were maintained in accordance with the guidelines of our institution's Ethical Committee and National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH Pub. No. 86-23 Rev. 1985).

The sliced specimens were stained with safranin O to assess the GAG contents. The GAG areas stained red by safranin O in the fibrocartilage layers in the AT, QT, PT, and ACL insertion were evaluated (Figure 1). Each red-stained GAG area was divided by the histologically measured width of the AT, QT, PT, and ACL insertion to define the average thickness of the red-stained GAG areas. All the parameters at different ages were compared between the AT, QT, PT, and ACL insertions. One-way analysis of variance (ANOVA) was used to evaluate the comparisons between AT, QT, PT, and ACL insertion. As a post hoc test, Tukey-Kramer was used. The level of significance was set at 5%.

RESULTS SECTION:

At 1 day of age, the GAG thickness in AT was higher than in ACL insertion ($p < 0.05$). At 1 week of age, the GAG thickness in QT was higher than in ACL insertion ($p < 0.05$). At 2 weeks of age, the GAG thickness in AT and PT was higher than in ACL insertion ($p < 0.05$). At 4 weeks of age, the GAG thickness in QT was higher than in ACL insertion ($p < 0.05$). At 6 weeks of age, there was no significant difference among the 4 groups. From 8 to 24 weeks of age, the GAG thickness in ACL was higher than in AT, QT, and PT insertion ($p < 0.05$).

DISCUSSION:

The GAG area at the ACL insertion was smaller than the AT, QT, and PT insertion in the early stage of development. However, the GAG area at the ACL insertion became larger than the AT, QT, and PT insertion as it matured.

The differences between the ACL insertion and the AT, QT, and PT insertion can also be due to the differences in the structures and mechanical environments. In the ACL, both ends are bone, whereas muscle is at one end of the AT, QT, and PT complexes. Muscle tension and traction in the growth period affect the AT, QT, and PT complexes. The ACL insertion may receive a greater load than AT, QT, and PT insertion as it matures.

This study had some limitations. First, because the skeletal growth of rabbits is complete at 6 months, we performed histological analyses until 6 months of age. Evaluations may be necessary after 6 months to analyze the fibrocartilage layer after the growth period. Second, mechanical analyses will be necessary to clarify the association of mechanical stresses.

SIGNIFICANCE/CLINICAL RELEVANCE:

Our results support the consideration of appropriate treatment strategies based on the insertion site, and the development of new treatment methods for the regeneration of the tendon–bone interface and insertions.

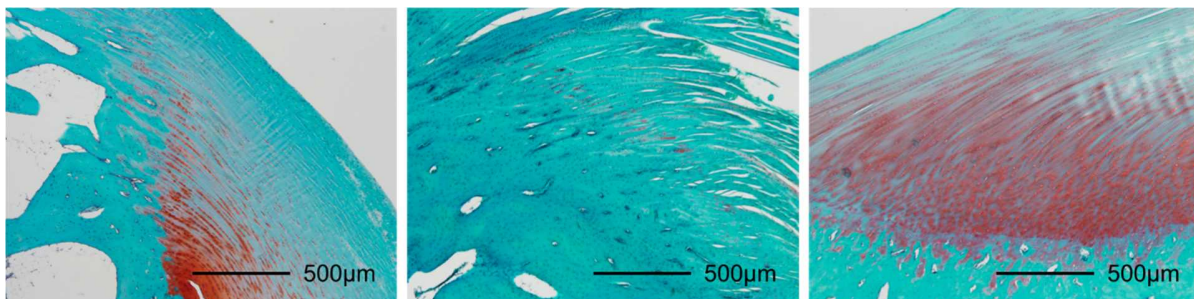


Figure 1. Histological sections of AT (left), QT (middle) and ACL (right) insertion at 24 weeks of age stained with safranin O (40×)