Differences in the Development of Fibrocartilage Layers in the Quadriceps Tendon and Patellar Tendon Insertions in Rabbits

A Quantitative Study

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Background: Differences in the development of fibrocartilage layers in quadriceps tendon (QT) and patellar tendon (PT) insertion sites are unclear. Because the mechanical environments for the QT and PT are different, the development of the QT and PT insertions may differ.

Purpose: To investigate differences in the development of fibrocartilage layers in the QT and PT insertion sites in rabbits through use of quantitative morphometric evaluations.

Study Design: Descriptive laboratory study.

Methods: This study included 54 male Japanese White rabbits. Animals were euthanized at ages 1 day and 1, 2, 3, 4, 6, 8, 12, and 24 weeks (n = 6 for each age). Chondrocyte number, proliferation, apoptosis, sex-determining region Y box 9 (Sox9)–positive rates, safranin O–stained glycosaminoglycan (GAG) areas, tidemark length, insertion width, and patellar length were evaluated and compared with the same parameters at age 24 weeks and between QT and PT insertion sites.

Results: Chondrocyte proliferation was low up to age 2 weeks for QT insertion and low up to 1 week for PT insertion. Chondrocyte apoptosis was high at 1 day and Sox9 expression was low up to 1 week for PT insertion. Sox9 expression was higher in QT than in PT insertion at age 12 weeks. The high chondrocyte count continued to age 1 day in PT insertion and up to 6 weeks in QT insertion. The chondrocyte number was higher in QT than in PT insertion at age 2 weeks. The period of thicker GAG lasted from 2 to 8 weeks in PT insertion and from 1 to 12 weeks in QT insertion. GAG thickness in QT insertion was higher than in PT insertion at age 4 and 12 weeks.

Conclusion: Development of fibrocartilage layers in QT and PT insertion sites was completed at age 24 weeks in rabbits. However, the period of high chondrocyte count and period of thicker GAG were longer in QT than in PT insertion up to 12 weeks.

Clinical Relevance: Development of fibrocartilage layers in QT and PT insertions differed in rabbits. Our results may contribute to the development of appropriate treatments based on age and the development of methods for regeneration of the insertion.

Keywords: development; quadriceps tendon insertion; patellar tendon insertion; fibrocartilage layers; glycosaminoglycan

Direct-type insertions such as quadriceps tendon (QT), patellar tendon (PT), and anterior cruciate ligament (ACL) include 4 transitional tissue layers: ligament or tendon, 2 fibrocartilage layers (unmineralized and mineralized), and bone.[19] Mechanical stress is reduced based on the various degrees of stiffness of these layers at the insertion.[19] A few studies have evaluated QT and PT insertion development.[3,9] From 2 to 6 weeks of age in rabbits, the fibrocartilaginous layer in the QT insertion became mature, and fibrocartilage and mineralized fibrocartilage became more distinct under qualitative analysis.[3] In another study, tenocyte proliferation in PT occurred at its highest levels during late fetal life and declined to very low levels by 2 weeks after birth in mice.[9] Knowledge of the differences in the development process and anatomic structure of the fibrocartilage layers in the QT and PT insertion sites is important for development of age-appropriate treatment and new treatment methods for the tendon-bone interface.

Glycosaminoglycan (GAG) in the fibrocartilage layers is an important load transmitter because of its tissue elasticity and its resistance to tensile, shear, and compressive stresses.[1,19] The development of fibrocartilage layers in the

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ACL tibial insertion, chondrocyte apoptosis, chondrocyte proliferation, sex-determining region Y box 9 (Sox9), and GAG have been investigated in rabbits.[11] The study found that chondrocyte proliferation and Sox9 expression increased up to 12 weeks of age, along with GAG production and tidemark length, in accordance with development of the ACL length and its insertion width.[11] Additionally, the development of fibrocartilage layers in the ACL insertion was complete at 12 weeks of age.[11]

However, differences in the development of fibrocartilage layers in the QT and PT insertions are unclear. Because the mechanical environments for the QT and PT insertions are different, development of the QT and PT insertions may differ. In the current study, we therefore investigated the differences in the development of fibrocartilage layers in QT and PT insertions in rabbits through use of quantitative morphometric evaluations. We hypothesized that the fibrocartilage layers in the QT and PT insertions would develop with age and that this development would differ between the insertion sites.

METHODS

Animal Preparation

This study included 54 male Japanese White rabbits. The animal species to be studied was determined based on previous reports.[11-14] We chose rabbits for this study because it is difficult to prepare tissue specimens using animals smaller than rabbits. Only male rabbits were included because many prior studies were conducted using male rabbits for histomorphometric analyses of the ACL and PT insertion, and also to eliminate the influence of female hormones.[7,11-14] Considering that this study was an evaluation of the development of fibrocartilage layers in QT and PT insertions, it could not be considered an in vitro study. Because skeletal growth is complete at 6 months in rabbits,[10] we set 24 weeks of age as the end of the evaluation period. A total of 6 animals at each age (1 day and 1, 2, 3, 4, 6, 8, 12, and 24 weeks) were euthanized by injection of an overdose of intravenous barbiturate (200 mg/kg, Somnopentyl; Kyoritsu Seiyaku Corp).

The rabbits were maintained in accordance with the guidelines of the institution's ethics committee and the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH Pub. No. 86-23 Rev. 1985). This study conformed with ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

Staining Method and Immunohistochemistry

A randomly chosen unilateral QT-patella-PT complex from each animal was fixed in 10% neutral-buffered formalin for 1 week. Then, the specimens from 2 to 24 weeks of age were decalcified in 10% ethylenediaminetetraacetic acid (EDTA; pH 7.4) from 7 to 12 weeks. After decalcification they were embedded in paraffin. Decalcification was not required for specimens from rabbits 1 day and 1 week of age. In all specimens, 5-μm slices of the QT-patella-PT complexes were made in the sagittal plane.

The sliced specimens were stained with hematoxylin and eosin and with safranin O to assess the histomorphologic features and GAG contents.[11-14] To detect proliferating cells, sliced specimens were stained with proliferating cell nuclear antigen (PCNA), using a Histofine SAB-PO (M) Kit (Nichirei Biosciences Inc) according to the manufacturer's instructions (Figure 1A).[11-14] An anti-PCNA monoclonal antibody (PC-10; Code No. M0879; Dako) and antibody diluent (Code No. S0809; Dako) were also used. To detect apoptotic cells, TUNEL (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick-end labeling) staining was performed using an Apoptag Plus Peroxidase In Situ Apoptosis Detection Kit (Merck Millipore) according to the manufacturer's instructions (Figure 1B).[11-14] To evaluate the developmental differentiation of chondrocytes, Sox9 staining was performed with a Histofine SAB-PO (R) Kit (Nichirei Biosciences Inc) and a Rabbit-to-Rabbit Blocking Reagent (ScyTek Laboratories Inc) according to the manufacturers' instructions and including an anti-Sox9 rabbit polyclonal antibody (Bioworld Technology Inc) (Figure 1C).[11]

Histomorphometric Analysis

Histomorphometric analyses were performed using a BX-51 light microscope (Olympus Optical Co Ltd).[11-14] The GAG areas stained red by safranin O in the fibrocartilage layers in the QT and PT insertion were evaluated (Figure 2). In specimens at 1 day and 1, 2, 3, and 4 weeks of age, the fibrocartilage layers in the QT and PT insertion were defined as lower density staining of cartilaginous tissue with round cells compared with the hyaline cartilage area continuous with articular cartilage by safranin O, according to the previous report.[11] The fibrocartilage layers were located between the ligament and the hyaline cartilage area continuous with the articular cartilage.[11] In the specimens older than 4 weeks of age, the fibrocartilage layers in the QT and PT insertion were defined as cartilage layers with round cells between the ligament and bone.[11-14] The tidemark length in the QT and PT insertion was measured as the sum-total length stained with hematoxylin and eosin. The

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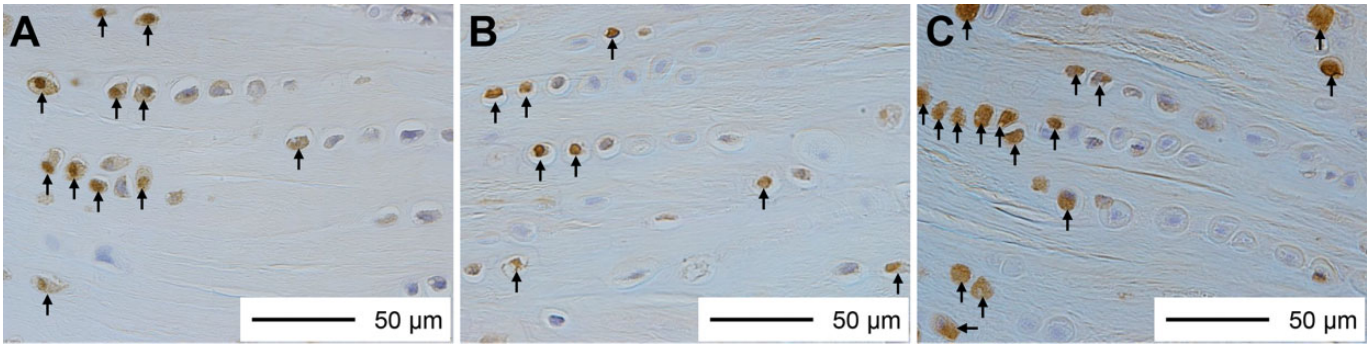

Figure 1. Histological sections. (A) PCNA staining (×400). (B) TUNEL staining (×400). (C) Sox9 staining (×400). Brown cells (arrows) are PCNA-positive, TUNEL-positive, or Sox9-positive chondrocytes, respectively. PCNA, proliferating cell nuclear antigen; Sox9: sex-determining region Y box 9; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick-end labeling.

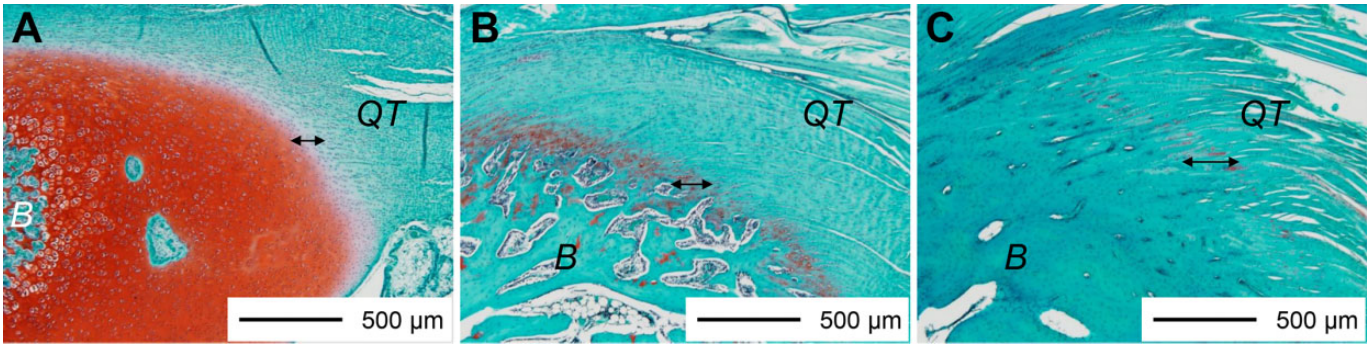

Figure 2. Representative safranin O–stained histological sections of quadriceps tendon insertions at the age of (A) 2 weeks, (B) 8 weeks, and (C) 24 weeks in rabbits (×40). Interfacial fibrocartilage layers (arrows) are evaluated. B, bone; QT, quadriceps tendon.

patellar length was defined histologically as the distance between the distal end of the fibrocartilage layers in the QT insertion and the proximal end of the fibrocartilage layers in the PT insertion. The histomorphometric analyses were conducted using Mac Scope software (Mitani Co). For each of the total numbers of chondrocytes, red-stained GAG area and tidemark length were divided by the histologically measured width of the QT or PT insertion to define the numbers of chondrocytes per width of insertion, the average thickness of the red-stained GAG areas, and the percentage of the tidemark length relative to the QT or PT insertion width, respectively. The TUNEL-, PCNA-, and Sox9-positive rates were calculated from the total numbers of chondrocytes in the safranin O–stained GAG areas in the fibrocartilage layers. All of the parameters at different ages were compared with those at age 24 weeks as well as between the QT and PT insertions.

Statistical Analysis

Normality was tested using the Shapiro-Wilks normality test for each parameter. One-way analysis of variance (ANOVA) or paired *t* test was used for evaluation of the time-dependent histological changes or the comparisons between the QT and PT insertion, respectively, if the normality of all variables for each parameter was confirmed. The Dunnett test was performed for factors that showed significant differences by ANOVA. If normality was not confirmed, the Kruskal-Wallis test and Bonferroni adjustment technique were used for the time-dependent histological changes, or the Wilcoxon signed-rank test was used for the QT and PT insertion. For time-dependent histological changes, we compared these parameters with those at age 24 weeks. The level of significance was set at 5%. SPSS Statistics Version 24.0 (IBM Corp) was used for all statistical analyses.

A power calculation was performed with a confidence level of 95% ($\alpha = .05$) and power ($1 - \beta$) of 80% using the POWER Procedure in SAS software (SAS Institute), according to previous studies.[13,14] Calculation of the smallest possible sample size that would produce a significant difference yielded an estimated sample size of 5 or 6 specimens per age group. We enrolled 6 specimens per age group to reduce the number of animals used.

RESULTS

Chondrocyte Immunohistochemistry

The chondrocyte proliferation rate in the QT insertion was significantly lower at ages 1 day and 1 and 2 weeks than at

TABLE 1
Results of Chondrocyte Immunohistochemistry[a]

	Chondrocyte Proliferation Rate			Chondrocyte Apoptosis Rate			Sox9-Positive Chondrocyte Rate		
Age[b]	QT	PT	*P*	QT	PT	*P*	QT	PT	*P*
1 d	0.36 ± 0.18[c]	0.34 ± 0.06[c]	.837	0.58 (0.49-0.63)	0.54 ± 0.08[c]	.600	0.34 ± 0.07	0.34 ± 0.08[c]	.956
1 wk	0.46 ± 0.12[c]	0.41 ± 0.07[c]	.446	0.58 ± 0.13	0.53 ± 0.07	.380	0.34 ± 0.18	0.34 ± 0.14[c]	.954
2 wk	0.40 ± 0.13[c]	0.46 ± 0.14	.322	0.52 ± 0.16	0.42 ± 0.09	.076	0.41 ± 0.04	0.40 ± 0.07	.926
3 wk	0.65 (0.56-0.70)	0.64 (0.56-0.65)	.173	0.37 ± 0.11	0.33 ± 0.07	.351	0.55 ± 0.10	0.56 ± 0.10	.576
4 wk	0.56 ± 0.05	0.63 ± 0.10	.107	0.44 ± 0.25	0.42 ± 0.21	.719	0.54 ± 0.11	0.54 ± 0.14	.923
6 wk	0.70 ± 0.09	0.62 ± 0.06	.145	0.43 ± 0.17	0.47 ± 0.12	.387	0.56 ± 0.10	0.52 ± 0.10	.118
8 wk	0.69 ± 0.09	0.63 ± 0.15	.144	0.52 ± 0.20	0.52 ± 0.25	.985	0.58 ± 0.05	0.55 ± 0.05	.332
12 wk	0.64 ± 0.04	0.58 ± 0.19	.422	0.54 ± 0.09	0.45 ± 0.07	.054	0.60 ± 0.10	0.47 ± 0.13	.007[d]
24 wk	0.63 ± 0.15	0.58 ± 0.13	.581	0.37 (0.36-0.45)	0.32 ± 0.11	.345	0.45 ± 0.09	0.52 ± 0.10	.206
Partial η^2/power	0.576/1.000	0.470/0.995	—	0.202/0.570	0.279/0.791	—	0.527/0.999	0.430/0.986	—

[a]Data are presented in percentages as the mean ± SD if normality was confirmed and as median (interquartile range) if normality was not confirmed. PT, patellar tendon; QT, quadriceps tendon; Sox9, sex-determining region Y box 9. Dashes indicate not applicable.
[b]n = 6 for each age group.
[c]Significant difference versus age 24 weeks ($P < .05$).
[d]Significant difference between QT and PT ($P < .05$).

24 weeks, and the rate in the PT insertion was significantly lower at ages 1 day and 1 week than at 24 weeks (Table 1, Figure 3A). The chondrocyte apoptosis rate in the PT insertion was higher at 1 day than at 24 weeks (Figure 3B). The Sox9-positive chondrocyte rate in the PT insertion was lower at ages 1 day and 1 week than at 24 weeks; also, compared with the PT insertion, this rate was higher at age 12 weeks in the QT insertion (Figure 3C).

Histomorphometric Analyses of Fibrocartilage Layers

The number of chondrocytes per width of insertion was significantly higher in the QT insertion at ages 1 day and 1, 2, 3, 4, and 6 weeks than at 24 weeks (Table 2). Regarding the PT insertion, this number was higher at 1 day than at 24 weeks (Figure 4A). The number of chondrocytes in the QT insertion was higher than in the PT insertion at age 2 weeks (Figure 4A). The thickness of the safranin O–stained GAG areas at ages 1, 2, 4, 6, 8, and 12 weeks was increased compared with that at 24 weeks in the QT insertion (Figure 4B). In the PT insertion, the thickness at ages 2, 3, 4, 6, and 8 weeks was increased compared with that at 24 weeks (Figure 4B). The thickness at age 3 weeks was greater in the PT than in the QT insertion, while the thickness at ages 4 and 12 weeks was greater in the QT than in the PT insertion (Figure 4B). The percentage of tidemark length relative to insertion width was lower at all ages from 1 day to 8 weeks compared with that at age 24 weeks, for both the QT and PT insertions (Figure 4C).

Histomorphometric Analyses of Insertion Width and Patellar Length

The width of the QT insertion was significantly lower at ages 1 day and 1, 2, 4, and 6 weeks than at 24 weeks, and the width of the PT insertion was significantly lower at ages 1 day and 1 week than at 24 weeks (Table 3, Figure 5A). The length of the patella at ages 1 day and 1, 2, 3, 4, and 6 weeks was significantly reduced compared with the length at age 24 weeks (Figure 5B).

DISCUSSION

This study analyzed the differences in the development of fibrocartilage layers in QT and PT insertion in rabbits through use of quantitative morphometric evaluations. The QT and PT insertion structures, including GAG thickness, width of insertion, length of patella, and tidemark, gradually developed up to age 24 weeks in the rabbits. However, the high chondrocyte count continued up to age 1 day in the PT insertion versus up to age 6 weeks in the QT insertion. The chondrocyte number was higher in the QT than in the PT insertion at age 2 weeks. The period of thicker GAG lasted from 2 to 8 weeks in the PT insertion and from 1 to 12 weeks in the QT insertion. The GAG thickness in the QT was higher than that in the PT insertion at age 4 and 12 weeks. It was more prominent in the QT insertion than in the PT insertion up to 12 weeks of age.

The period of high chondrocyte count and period of thicker GAG were more prominent in the QT versus the PT insertion up to 12 weeks. The differences may be due to the different mechanical stresses that the structures received. The QT insertion is directly connected to the quadriceps muscle and may be exposed to a greater tensile environment than the PT insertion. Rabbits undergo gait and skeletal growth up to age 12 weeks; therefore, the QT undergoes greater tensile stresses than the PT insertion. A real-time ultrasound elastography investigation revealed that the QT was stiffer elastic than the PT in humans.[16] Under gradual elongation of the PT using external fixation for 4 weeks in rabbits, the chondrocyte proliferation and GAG thickness was found to have increased.[14] Mechanical

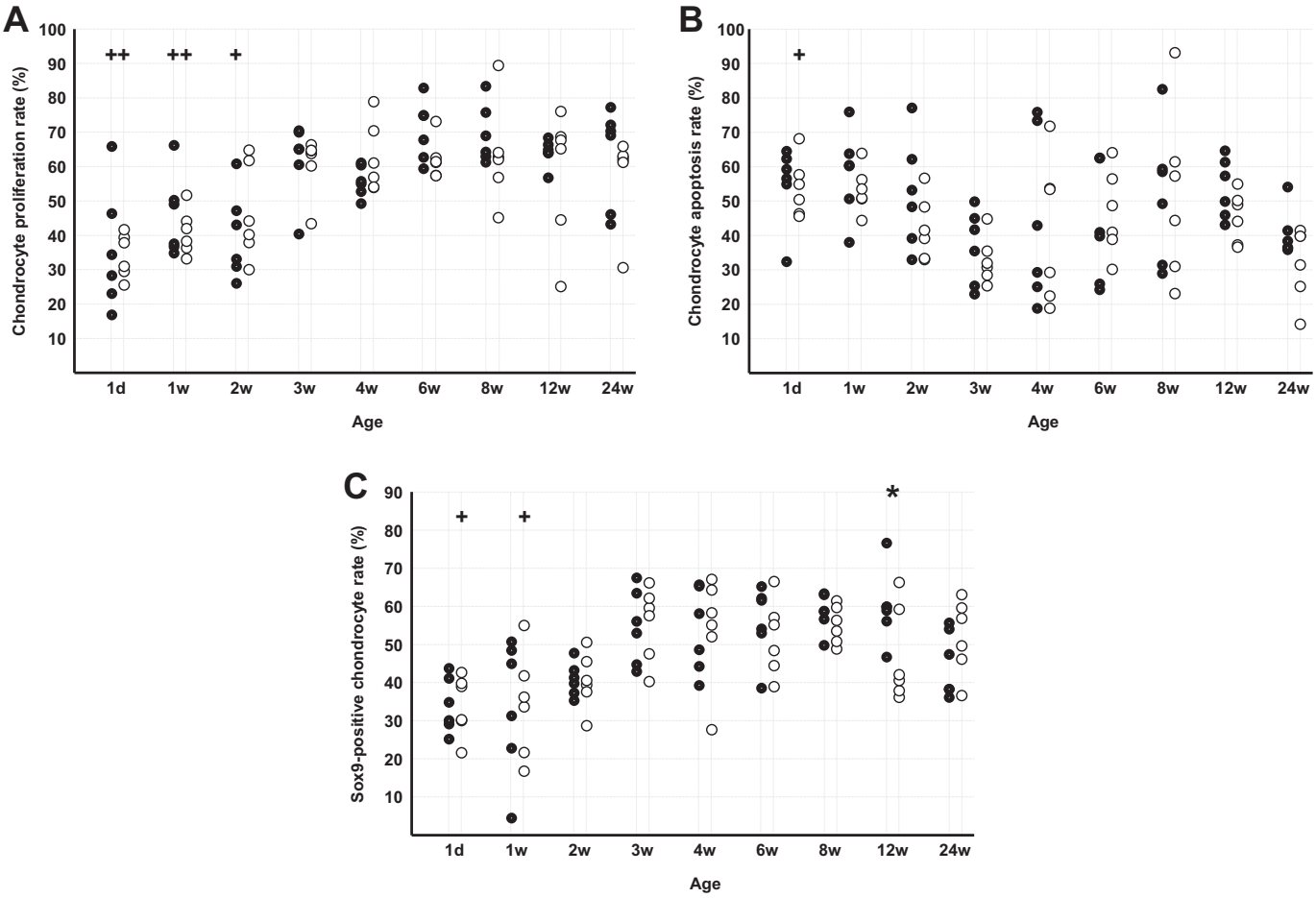

Figure 3. (A) Chondrocyte proliferation rate, (B) chondrocyte apoptosis rate, and (C) sex-determining region Y box 9 (Sox9)–positive chondrocyte rate. •, quadriceps tendon; ○, patellar tendon; $^{+}$Significant difference versus age 24 weeks ($P < .05$); *Significant difference between quadriceps tendon and patellar tendon ($P < .05$).

TABLE 2
Results of Histomorphometric Analyses of Fibrocartilage Layers[a]

	Numbers of Chondrocytes/Width of Insertion, n/μm			Thickness of Safranin O–Stained Glycosaminoglycan Areas, μm			Percentage of Tidemark Length		
Age[b]	QT	PT	*P*	QT	PT	*P*	QT	PT	*P*
1 d	0.22 ± 0.06[c]	0.31 ± 0.17[c]	.170	60.4 ± 22.0	47.4 ± 23.5	.109	0[c]	0[c]	—
1 wk	0.20 ± 0.05[c]	0.17 ± 0.10	.400	99.9 (59.8-162.7)[c]	79.3 ± 38.0	.345	0[c]	0[c]	—
2 wk	0.18 ± 0.05[c]	0.13 ± 0.05	.024[d]	98.8 ± 27.2[c]	132.8 ± 69.8[c]	.157	0[c]	0[c]	—
3 wk	0.17 ± 0.07[c]	0.17 ± 0.03	.884	74.6 ± 23.5	97.9 ± 23.0[c]	.045[d]	0[c]	0[c]	—
4 wk	0.19 ± 0.07[c]	0.18 ± 0.12	.885	132.2 ± 71.2[c]	75.8 (69.5-128.7)[c]	.046[d]	0[c]	0[c]	—
6 wk	0.26 ± 0.10[c]	0.24 ± 0.12	.694	102.3 (86.7-156.8)[c]	100.7 (82.1-151.4)[c]	.600	0[c]	0[c]	—
8 wk	0.11 ± 0.03	0.13 ± 0.04	.446	109.0 ± 31.4[c]	97.8 ± 18.1[c]	.501	0[c]	0[c]	—
12 wk	0.12 (0.11-0.15)	0.14 ± 0.04	.753	90.6 ± 16.4[c]	54.7 ± 13.7	.002[d]	35.3 ± 15.9	23.0 ± 18.4	.162
24 wk	0.07 ± 0.04	0.10 ± 0.06	.436	58.0 ± 14.7	43.9 ± 18.9	.229	64.1 ± 9.8	49.3 ± 17.4	.160
Partial η^2/power	0.483/0.997	0.353/0.927	—	0.248/0.710	0.381/0.956	—	0.936/1.000	0.817/1.000	—

[a]Data are presented as the mean ± SD if normality was confirmed and as median (interquartile range) if normality was not confirmed. PT, patellar tendon; QT, quadriceps tendon. Dashes indicate not applicable.
[b]n = 6 for each age group.
[c]Significant difference versus age 24 weeks ($P < .05$).
[d]Significant difference between QT and PT ($P < .05$).

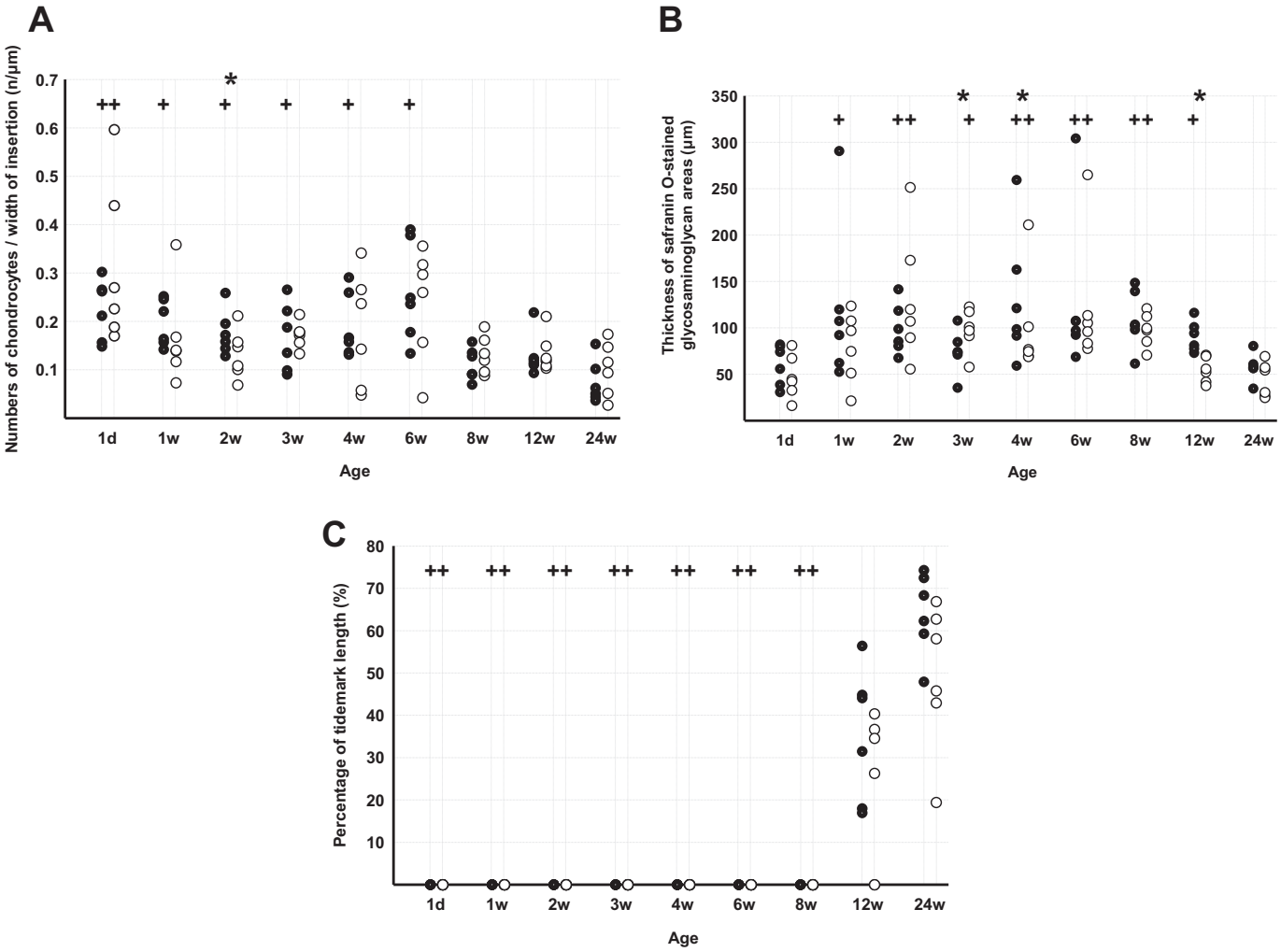

Figure 4. (A) Number of chondrocytes per width of insertion. (B) Thickness of safranin O–stained glycosaminoglycan areas. (C) Percentage of tidemark length. •, quadriceps tendon; ○, patellar tendon; $^{+}$Significant difference versus age 24 weeks ($P < .05$); *Significant difference between quadriceps tendon and patellar tendon ($P < .05$).

TABLE 3
Results of Histomorphometric Analyses of Insertion Width and Patellar Length[a]

	Insertion Width			
Age[b]	QT	PT	*P*	Patellar Length
1 d	0.90 (0.79-1.10)[c]	0.82 ± 0.23[c]	.116	2.39 ± 0.28[c]
1 wk	1.31 ± 0.29[c]	1.49 (1.35-1.55)[c]	.686	3.99 ± 0.43[c]
2 wk	1.61 ± 0.25[c]	1.78 ± 0.21	.127	4.34 ± 1.67[c]
3 wk	2.20 ± 0.99	1.62 ± 0.16	.167	4.40 ± 0.70[c]
4 wk	1.89 ± 0.31[c]	1.96 ± 0.50	.615	5.32 ± 0.98[c]
6 wk	1.84 ± 0.72[c]	1.97 ± 0.86	.085	4.18 (4.00-4.74)[c]
8 wk	2.84 ± 0.60	2.12 ± 0.30	.083	6.90 ± 0.59
12 wk	3.06 ± 0.73	2.82 ± 0.47	.575	8.81 ± 0.60
24 wk	3.27 ± 0.69	2.66 ± 1.19	.213	9.84 (8.91-10.05)
Partial η^2/power	0.655/1.000	0.553/1.000	—	0.900/1.000

[a]Data are presented in millimeters as the mean ± SD if normality was confirmed and as median (interquartile range) if normality was not confirmed. PT, patellar tendon; QT, quadriceps tendon. Dash indicates not applicable.
[b]n = 6 for each age group.
[c]Significant difference versus age 24 weeks ($P < .05$).

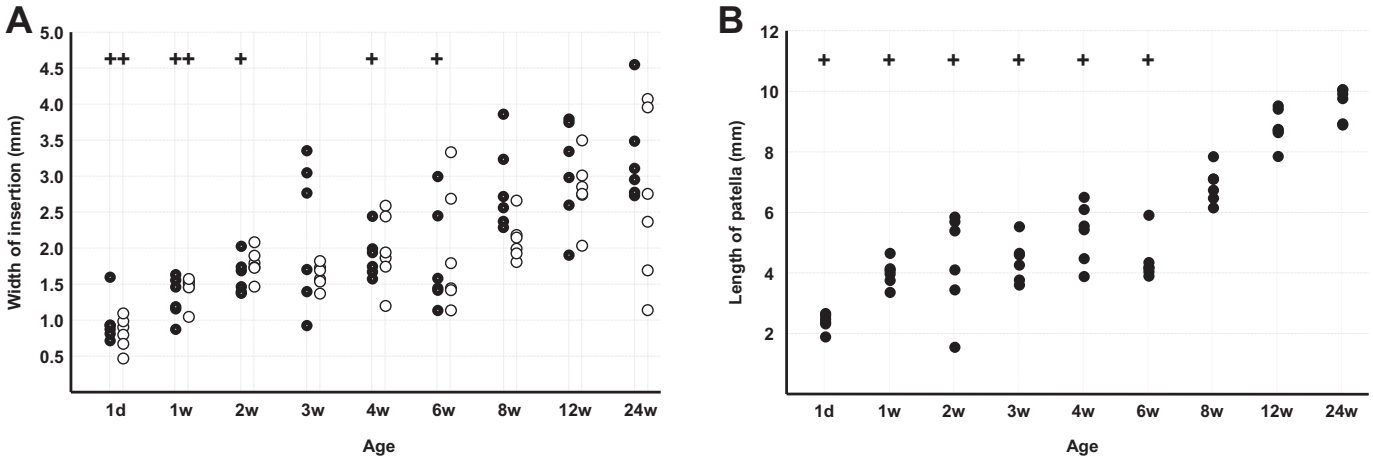

Figure 5. (A) Insertion widths for the quadriceps and patellar tendons and (B) patellar length. •, quadriceps tendon; ○, patellar tendon; $^{+}$Significant difference versus age 24 weeks ($P < .05$).

stress has been reported as a factor for the development of the fibrocartilage layers in the insertions.[2,18] Therefore, developmental differences in the QT and PT insertions might be due to their disparate mechanical environments.

The QT and PT insertion structures, including the insertion width, length of the patella, and tidemark, gradually developed to age 12 weeks, similar to the development of the ACL insertion.[11] Although the number of chondrocytes and the GAG thickness increased with time in the ACL insertion, in the QT and PT insertions, the high chondrocyte count lasted up to age 6 weeks and then decreased, and the period of thicker GAG lasted from ages 1 week to 12 weeks, then decreased. The differences between the ACL insertion and the QT and PT insertions can also be due to the differences in the structures and mechanical environments. In the ACL, both ends are bone, whereas there is muscle at 1 end of the QT and PT complexes. The QT and PT complexes are affected by muscle tension as well as traction in the growth period and may receive greater tensile stresses than the ACL insertion.

In this study, the fibrocartilage layers in the QT and PT insertions developed up to age 24 weeks in rabbits. In a previous study on rabbits, the fibrocartilage layers in the QT insertion qualitatively became mature, such as forming a mineralized fibrocartilage layer from 2 to 6 weeks of age.[3] The results of this previous study support our results. In our study, the chondrocyte proliferation was low up to age 2 weeks in the QT insertion and up to age 1 week in the PT insertion. The chondrocyte apoptosis was high at age 1 day, and Sox9 expression was low up to age 1 week in the PT insertion. In a previous study on mice, tenocyte cell proliferation in PT occurred at its highest levels during late fetal life and declined to very low levels by 2 weeks after birth.[9] We did not analyze a late fetal phase, and the previous report did not analyze animals after 2 weeks of age. However, chondrocyte proliferation may be low during the early period after birth in the QT and PT insertions, as mechanical stress to the QT and PT insertions from gait, muscle, and skeletal growth may be low during this period.

Clinically, insertional tendinopathies such as QT and PT rupture and jumper's knee are difficult to repair and treat.[6,17] The QT and PT, including part of the patella, are used as grafts in ACL reconstructions[4,8] and are difficult to regenerate.[5,15] Our results suggest consideration of appropriate treatment strategies based on age and the development of new treatment methods for regeneration of the tendon-bone interface and insertions.

This study has some limitations. First, because the skeletal growth of rabbits is complete at 6 months,[10] 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, to clarify the association of mechanical stresses, mechanical analyses will be necessary. Third, because we evaluated only the phenotype in this study, investigation of the pathways and network of signaling systems is needed.

CONCLUSION

The development of fibrocartilage layers in the QT and PT insertions was complete at age 24 weeks in rabbits. Although the high chondrocyte count lasted up to age 6 weeks and then decreased and the period of thicker GAG lasted up to 12 weeks and then decreased, both were more prominent in the QT compared with the PT insertion. Thus, development of fibrocartilage layers in rabbits was different between the QT and PT insertions.

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