The Effect of Cyclic Loading on Rotated Bone-Tendon-Bone Anterior Cruciate Ligament Graft Constructs

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Background: Single-incision anterior cruciate ligament reconstruction with a bone–patellar tendon–bone construct is commonly performed with 180° rotation of the graft. It has been hypothesized that further rotation of the graft to 540° can effectively shorten the graft to address graft length–tunnel mismatch. Initial biomechanical failure characteristics of rotated constructs have been reported, but cyclic loading of tendons has not been performed.

Hypothesis: Graft rotation affects the biomechanical properties of the construct.

Study Design: Controlled laboratory study.

Methods: Thirty-five bone–patellar tendon–bone composite porcine right knee specimens were randomized into 3 groups and were externally rotated to 0°, 180°, or 540°. Each group was then cyclically loaded in an artificial synovial fluid medium between 50 and 250 N for 5000 cycles, loaded between 50 and 500 N for an additional 5000 cycles, and finally subjected to load-to-failure testing.

Results: Graft rotation shortened constructs by 1.7 ± 0.8 mm at 180° of rotation and 7.6 ± 2.0 mm at 540° of rotation ($P < .01$). There was a statistically significant increase in strain during cyclic loading at 540°. No significant differences in maximum load, yield stress, yield strain, or modulus of elasticity were detected in single-cycle load-to-failure testing after cyclic loading.

Conclusion: Rotation of bone–patellar tendon–bone constructs to 540° predictably shortens the effective graft length at the expense of increased strain with cyclic loading at stresses equivalent to walking and running.

Clinical Relevance: Although rotation to 540° potentially addresses graft length–tunnel mismatch, further clinical evaluation is required to evaluate the impact of increased strain on knee laxity and to determine the effects of physiologic loading of rotated bone–patellar tendon–bone constructs in vivo.

Keywords: anterior cruciate ligament (ACL); bone–patellar tendon–bone (BPTB); graft rotation; twist; biomechanics

Rotation of bone–patellar tendon–bone (BPTB) grafts during ACL reconstruction has been advocated for many reasons. Rotation of 180° has been theorized to improve fixation within the tibial tunnel[3,34,42] and to prevent wear of the tendon at the edges of the tibial tunnel.[19] External rotation may reproduce the natural rotation of the ACL[1,11,23,34] and may improve graft isometry.[5,12] More recently, rotation of the BPTB graft up to 540° has been used to shorten the graft to address graft length–tunnel mismatch.[2,39]

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No potential conflict of interest declared.

The American Journal of Sports Medicine, Vol. 34, No. 9
DOI: 10.1177/0363546506287741

The impact of rotation on the biomechanical properties of the BPTB construct, however, is largely undetermined. Several authors have found no effect of rotation.[22,26,27] Using intraoperative measurements, Arnold et al[1] cited changes in the force on the ACL with variable rotation. Cooper et al[7,8] reported a statistically significant increase in ultimate load to failure in graft rotation from 0° to 90° ($P < .05$), with no additional increase with further rotation to 180°. Hame et al[16] measured decreased AP laxity and increased graft tension at 90° and 180° of rotation. In our previous report, single-cycle load-to-failure testing of rotated grafts up to 540° found no difference in ultimate failure strength but noted a trend toward increasing strain at higher degrees of rotation.[39]

The purpose of this study was to examine the biomechanical effects of various amounts of rotation on cyclic-loaded BPTB grafts. To our knowledge, a cyclic loading analysis of rotated grafts has not been previously reported.

A secondary goal was to quantify the shortening achieved with external rotation of the graft. Our null hypothesis was that graft rotation does not meaningfully affect yield strength or modulus of elasticity of the construct.

MATERIALS AND METHODS

Fifty nonmatched BPTB porcine specimens were obtained from a local meat packing plant from animals processed for food sale at the time of slaughter (Peoria Packing Company, Chicago, Ill). All specimens were obtained from the hindlimbs and were right legs. This choice allowed accurate control of the direction of rotation. A previously validated protocol was used for preparation of the specimens.[39] Grafts were harvested using a standard surgical technique, and the patellar tendon was trimmed to 10 mm. The tibial tubercle was harvested from the tibia with an oscillating saw. The patella was left intact. After harvest, specimens were frozen in double-bagged saline-soaked gauze until ready for testing.[4]

A pilot study using 10 specimens was performed to standardize the technique of potting and testing and to perform an initial power analysis. During actual testing, 5 specimens that sustained fracture of the bone plugs or failure of the mold were excluded from the data. The data from the remaining 35 specimens were tested without technical failure and were included in this study.

Tendons were randomized to 1 of 3 groups with external graft rotation of 0°, 180°, or 540°. On the day of testing, specimens were allowed to thaw at room temperature for 12 hours. The cross-sectional area of the tendon was determined using a previously described area micrometer method.[13] Geometrical cross-sectional area was measured at 3 separate areas. Plastic tubing was placed around the patellar tendon, and 2 screws were placed through both the patella and the tibial tubercle in the coronal plane, perpendicular to the tendon (Figure 1). Testing was conducted using clamps for graft fixation modeled after those used by Cooper[7] and in a similar fashion to Verma et al.[39] The patella and tibial tubercle were potted using acrylic polymer cement (Isocryl, Lang Dental, Chicago, Ill). The base of the plastic tubing was potted with the tibial tubercle forming a sealed container around the tendon (Figure 2). The patellar tendon was protected from thermal injury, and a phosphate-buffered saline spray was used to keep the tendon moist during the potting process.

Biomechanical testing was conducted using an Instron testing device (Instron, Canton, Mass) with a 5000-N load cell. The clamps were attached to both the base and load cell using 1-in threaded steel rods. By sliding the molds within the clamps, appropriate vertical and rotational alignment was achieved. Rotation was achieved by removing the mold from the clamp, rotating the desired amount, and replacing the mold within the clamp. All specimens were rotated externally. Graft length before and after rotation was determined using the Instron machine in a load control mode with a set point of 5 N to remove tissue crimp and improve consistency of measurement. The clamp-to-clamp distance was measured with a digital caliper (Starrett, Athol, Mass),

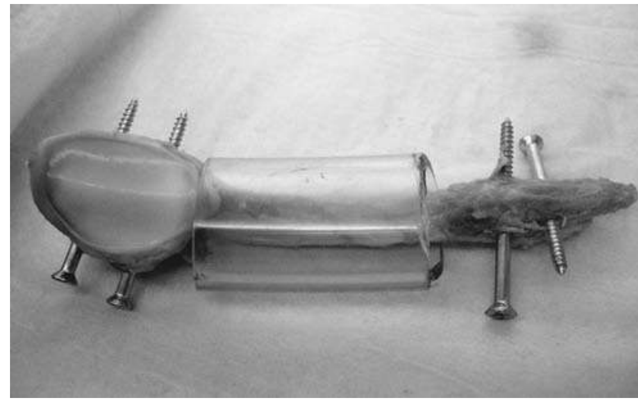

Figure 1. Grafts were harvested from porcine specimens using a standard surgical technique, and the patellar tendon was trimmed to 10 mm. Plastic tubing was placed around the patellar tendon, and 2 screws were placed through both the patella and the tibial tubercle in the coronal plane, perpendicular to the tendon.

Figure 2. The patella and tibial tubercle were potted with the base of the plastic tubing forming a sealed container around the tendon. This tubing was filled with artificial synovial fluid to immerse the tendon for the remainder of the experiment. In this way, desiccation of the tendon was prevented during extended testing times.

and a measure of the embedded tendon within each mold was added to the measurement. This process provided a measurement of the tendon length itself without inclusion of the bone blocks and permitted calculation of the graft elongation ratio (tendon strain).

Because the testing protocol was longer than 5 hours, a novel method of preventing desiccation of the tendon over time was instituted. The plastic tubing surrounding the tendon was filled with artificial synovial fluid (BioEngineering Solutions, Chicago, Ill) to immerse the tendon for the remainder of the experiment. A saline-soaked sponge was loosely wrapped around the inferior pole of the patella to prevent the uppermost part of the tendon, inevitably pulled out of the synovial solution, from drying out.

Preconditioning of each specimen was performed by loading the tendon to 80 N over 30 seconds in load control mode.[31] This load was held for 10 seconds. The feedback control (proportional integral derivative) of the Instron machine was individually calibrated for each sample.

Cyclic loading of each tendon was initially performed in load control mode in a sinusoidal wave between 50 and 250 N for 5000 cycles at 0.5 Hz. This mode approximated forces seen in the ACL during walking. The tendon was then cyclically loaded in a sinusoidal wave between 100 and 500 N for an additional 5000 cycles at 1.0 Hz to approximate forces seen in the ACL during running. Mean initial cross-sectional areas were used to calculate stress and strain at the peaks and troughs of each wave. Loading was performed in the linear portion of the stress-strain curve as verified through the pilot study. This loading allowed a calculation of the "instantaneous" modulus of elasticity using the difference in stress and strain for 1 cycle.

Finally, single tensile loading to failure was performed for each tendon. In this phase, an elongation rate of 5 cm/s was used to approximate the 100% per second rate previously described.[4,30] This rate was chosen to optimize the chance of obtaining a midsubstance failure (Figure 3). A stress-strain curve was generated for each specimen tested (Figure 4).

A power analysis was performed. The use of 10 specimens per group (N = 30) ensured 87% power for detecting a large effect (f = 0.8) based on 1-way analysis of variance of the square root transformed load-to-failure measurements.[6]

A randomization order was used to randomize the testing with regards to rotation. Data were stored using an IBM data acquisition computer system, and statistical analysis was performed with SPSS (version 11.5, SPSS Inc, Chicago, Ill). Because the histograms indicated the data were not in a normal distribution, the nonparametric Kruskal-Wallis statistical test was used. Post hoc analyses were performed with a Mann-Whitney test to compare individual groups if significance was noted within the Kruskal-Wallis testing.

RESULTS

There were no significant differences in cross-sectional areas or lengths between tendon groups. Mean porcine tendon length was 54.0 ± 2.4 mm. By externally rotating the grafts,

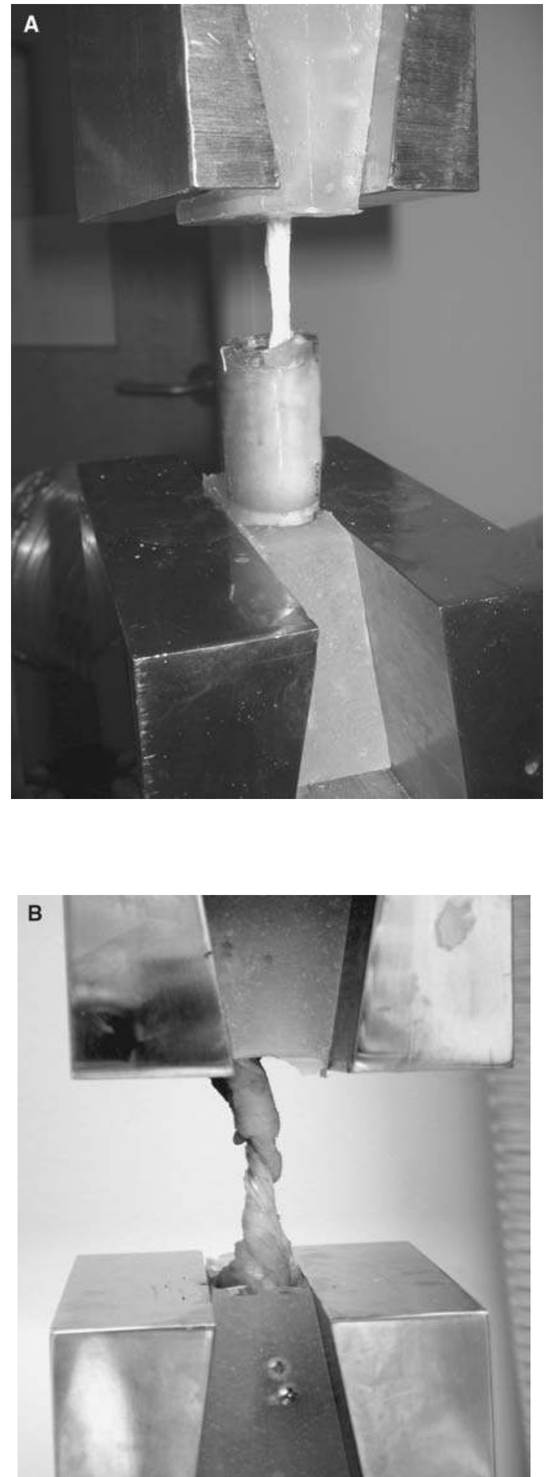

Figure 3. Constructs were tested at 0°, 180°, and 540°. A, graft at 0° rotation after midsubstance failure. B, construct at 540° of rotation. Tubing and synovial fluid were removed for this picture to allow visualization of the twist in the graft. Measurement of the graft elongation ratio (strain) based on the total tendon length was calculated over time. After cyclic loading for 10 000 cycles, a single-cycle load-to-failure test was performed.

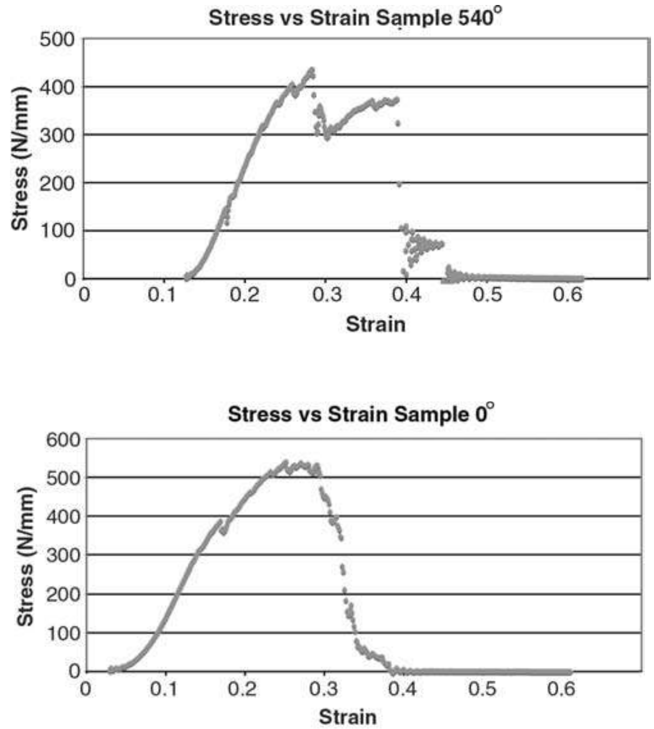

Figure 4. Sample stress-strain curves were generated for each specimen tested.

constructs shortened a mean of 1.71 ± 0.8 mm (3.0% ± 1.5%) at 180° and 7.56 ± 2.0 mm (13% ± 3.5%) at 540°. There were no significant differences in tendon lengths between 0° and 180° of rotation. Between the 540° and the 180° groups and between 540° and 0° groups, significant differences in final tendon lengths were noted ($P < .01$).

All tendon failures were midsubstance and occurred during load-to-failure testing after cyclic loading. Table 1 includes measurements of the mean maximal and minimal strain at the 5000th cycle and at the 10 000th cycle for each group. It also includes a measurement of the modulus of elasticity as calculated above for each of the groups at the end of the cyclical loading. At the 5000th cycle of cycling between 50 and 250 N, there were no significant differences between modulus of elasticity. An increased maximum and minimum strain in the 540° group was noted compared with both the 0° and 180° groups ($P < .001$) (Figure 5). At the 5000th cycle of cycling between 100 and 500 N, there were again no significant differences between modulus of elasticity. Increased maximum and minimum strain was again noted compared with both the 0° and 540° groups ($P < .004$).

Table 2 provides data from the end static tensile loading to failure for each group. Yield stress was lower in the 540° group but did not reach statistical significance (46.0 ± 5.7 N/mm in the 540° group compared with 53.7 ± 5.4 N/mm in the 0° group). There was a statistically significant difference in yield strain between the 540° group and the 0° and 180° groups. There were no significant differences in modulus of elasticity, maximum load, yield stress, or ultimate stress or strain between groups.

DISCUSSION

Although single-cycle load-to-failure testing of ACL grafts provides information regarding the biomechanical properties at the time of implantation, these tests do not address the mechanical behavior of the graft construct during healing. During the initial healing period, the environment is one of submaximal cyclic loading. Repetitively loading the ACL graft more likely approximates this early recovery period and was chosen for this study to provide a more accurate examination of the influence of rotation on graft properties.[4,35]

Cyclic loading has been advocated by multiple authors.[†] Among other investigations, it has been used to evaluate various methods of graft fixation,[20,21,24,36,38,41] graft elongation in the early recovery period,[17,37] initial graft tension,[31] and the effects of radiation.[9] To our knowledge, cyclic loading of rotated BPTB tendons has not been previously reported.

No standard protocol has been defined to perform cyclic loading of ACL grafts. Beynnon and Amis[4] recommended loading the knee to 1000 load cycles at 150 N of displacement to evaluate the initial cyclic loading response. No specific rationale was provided for this value. Jarvinen et al[20] chose to load grafts for 1500 loading cycles between 50 and 200 N at 0.5 Hz. Weiler et al[41] applied forces from 50 to 300 N, gradually adding force until tendon failure. Numazaki et al[31] performed a force-relaxation test for 5000 cycles with enough force to stretch the graft 2 mm with each cycle. Kousa et al[21] repeated 100 cycles at increasing loads up to 850 N. Honl et al[18] tested constructs at 300 N for 60 000 cycles to represent the equivalent activity for 4 weeks.

In this study, after preconditioning, we chose to test each specimen for 10 000 cycles at 2 different loading forces. This value is an order of magnitude larger than that recommended by Beynnon and Amis[4] and was chosen to maximize loading of the graft construct. The BPTB constructs were loaded between 50 and 250 N for 5000 cycles to simulate forces on the ACL during walking. The construct was then loaded for 5000 cycles between 100 and 500 N to simulate forces on the ACL during strenuous physical therapy or jogging. Finally, constructs were subjected to a single-cycle load-to-failure testing to gain further data about the effects of cyclic loading.

The extended testing times within this study required a novel method of preventing drying out of the BPTB construct. The tendon was immersed in an artificial synovial fluid throughout the testing protocol. The ACL, usually an extrasynovial ligament, would normally be subjected to an environment of hematoma and synovial fluid during the immediate postoperative period. Thus, the synovial fluid may also provide a more physiologic environment.

A porcine model was chosen for this study because of the limited availability of younger human cadaveric specimens. The large variation in age, sex, and physical condition that

[†]References 4, 9, 17, 20, 21, 24, 31, 35-38, 41.

TABLE 1
Cyclic Loading Data

Rotation	Maximum Strain		Modulus, N/mm	
	5000th Cycle	10 000th Cycle	5000th Cycle	10 000th Cycle
0°	0.06 ± 0.01	0.09 ± 0.01	216 ± 18	299 ± 28
180°	0.07 ± 0.01	0.10 ± 0.02	208 ± 30	287 ± 32
540°	0.16 ± 0.05[a]	0.18 ± 0.06[a]	246 ± 36	297 ± 33
P	.001	.004	.43	.93

[a]Significantly different from other groups.

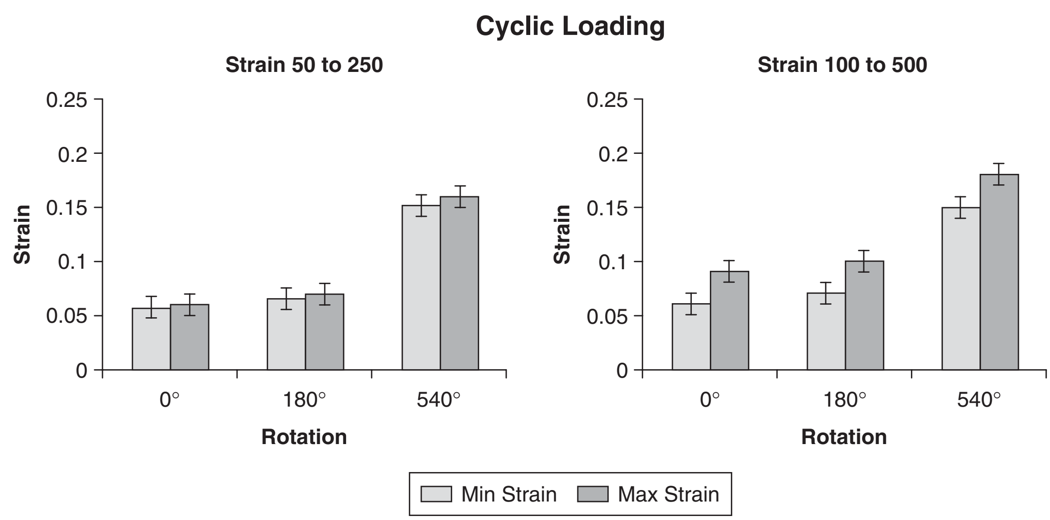

Figure 5. Strain during cyclic loading. A statistically significant increased strain was noted at 540° rotation compared with both 0° and 180°.

TABLE 2
Single-Cycle Load-to-Failure Testing

Rotation	Yield Strain	Maximum Load, N	Maximum Stress, N/mm	Modulus, N/mm
0°	0.23 ± 0.07	2269 ± 275	53.7 ± 5.4	342 ± 38
180°	0.22 ± 0.02	2150 ± 290	51.5 ± 6.6	315 ± 45
540°	0.30 ± 0.07	1937 ± 233	46.0 ± 5.7	319 ± 36
P	.18	.17	.13	.43

would be present in human specimens would have been unacceptable. The porcine model has been used extensively in the past to study the ACL, and the biomechanical properties of the porcine soft tissue have been found to closely approximate those of humans.[14,15,28]

Calculations of strain were based on the initial and final length of the tendon itself, excluding bone blocks. Nonuniform deformation immediately adjacent to gripped (ie, embedded) tendon in our study decreased the precision of using clamp-to-clamp distance as a determinant of deformation. Even so, all specimens tested demonstrated midsample rupture, and our methods were based on past investigations.[7,9,27,39] The large strains and sample lengths tended to minimize this confounding effect on clamp-to-clamp measurement, which was used in this study more for comparison purposes between rotated and nonrotated tendons and less as a determinant of true tendon mechanical/structural properties.

Graft Shortening

Mean porcine tendon length was 54.0 ± 2.4 mm and approximated that of human patellar tendon grafts.[10,40] With 540° of external rotation, a mean shortening of 7.6 mm was observed. This finding represented a mean of 13% of initial tendon length. There was minimal shortening at 180°. These results are consistent with those seen by Verma et al[39] and confirm the relationship between graft shortening and the degree of rotation.[2]

Rotation of BPTB Constructs

In this study, no significant differences were noted between tendons rotated up to 540° during load-to-failure testing after cyclic loading. During cyclic loading tests, however, there was an increased strain (percentage elongation) in the group of tendons rotated 540° compared with lesser degrees of rotation ($P < .001$). A statistically increased strain in the tendons rotated 540° was also noted during load-to-failure testing after cyclic loading. A power analysis was performed to judge the number of samples needed to detect differences in yield strength between groups. This study had an 80% chance of detecting a 20% difference in groups for yield strength.

These results are similar to those from our previous report of single-cycle load-to-failure testing of rotated grafts up to 540°.[39] In both studies, an increased strain was noted with rotation past 180°. The elevated strain seen could theoretically lead to increased knee laxity at physiologic loads.

Our results are also consistent with a recent clinical report by Verma et al[40] that noted a 23% incidence of KT-1000 arthrometer values between 3 and 5 mm in a subgroup of patients with 540° rotation at 34 months postoperatively. In that study, however, no clinical difference could be detected between reconstruction with a free tibial bone block and 540°.[40] Stability of grafts was maintained at 1-year follow-up in an earlier clinical study as well.[2] Further clinical research is necessary to evaluate these results.

The findings of this study are limited by the fact that this was an in vitro study. Testing methods did not include angular or rotational forces and do not necessarily reflect forces encountered by the graft in vivo. Although the cyclic loading analysis in this study provided a better evaluation of the initial healing environment, it is not known how the biologic processes of remodeling and revascularization affect the late biomechanical properties of the graft. Further studies are needed to more fully evaluate these possibilities.

A less than 5% increase in the modulus of elasticity in the tendon over the 5000 cycles of loading was seen in each sample. This may be an artifact of the testing conditions or may simply be owing to the viscoelastic nature of the tendon and the strain-rate dependence.

Although the concept of twisting ACL grafts is relatively new, the study of the physical properties of twisted structures has been a core component within the textile sciences for several decades.[32,33] The fiber industry routinely twists fibrous structures to not only produce yarns, cords, and ropes but also to modify the mechanical properties of their fibers. In this way, high-performance fibers can be custom designed to address specific needs. Kevlar is one example of such a fiber.[33]

The individual fibers within twisted structures can interact with each other in a manner different from their parallel configuration. Individual fibers reinforce each other as stress is transferred between filaments. Loads are transferred from fiber to fiber in these twisted structures, potentially altering their mechanics. Twisted structures have been found to have altered biomechanical properties depending on the twist level, the lateral contraction ratio, and the baseline fiber stress-strain properties (among other variables).[32]

More recently, mathematical models have been used to describe the elastic behavior of twisted fibers.[29,32] The addition of twist to yarn has been found to decrease the modulus of elasticity.[29,32,33] In a simplified model, modulus decreases proportional to cosine squared of the twist angle.[33] As the surface twist angle increases, the modulus further decreases. This decrease can lead to an increased ultimate strain.[33]

Strain was used in this study to describe tendon elongation. Localized strain experienced by the individual fibers was not specifically measured. If tendons behave similarly to yarn, rotation of the graft will increase the twist angle, which will differentially increase the loading forces within the fibers of the rotated graft when subjected to uniaxial stresses. The actual strain in the individual fibers will, thus, be larger than the observed graft strain because of these altered internal stresses. However, the degree to which annular fibrils in tendons undergoing twisting mimic the behavior found in previous investigations of yarn is not known. Alterations in the stress field across the tendon fibers after rotation compared with unrotated tendons under similar uniaxial loading are beyond the scope of this study but become important as cyclic loading approaches the ultimate stress of fibers and tendons. This is the subject of future structure-function investigations.

The strength of twisted structures is more difficult to predict than is their parallel fiber counterparts.[32] Strength is dictated by the weakest cross section of the structure as well as by fragmentation processes (influenced by the geometric and surface properties of the filaments).[32] Nonetheless, recent experiments have found that the strength of some industrial twisted fibrous structures can be improved with a slight twist.[33] After reaching an optimal twist level, further twisting can decrease the tensile strength of the structure.[33]

No relationship has yet been established between twisted structures within the textile sciences and twisted human tendons or ligaments. Even so, Verma et al[39] noted a decreased modulus of elasticity and increased strain with increasing rotation up to 540°, although these results were not clinically significant. Cooper[7] reported a statistically significant increase in ultimate load to failure (strength) in graft rotation from 0° to 90° ($P < .05$), with no additional increase with further rotation to 180°. Millett et al[25] described decreased initial tensile strength and stiffness in braided hamstring grafts. All of these studies are consistent with the decreased modulus, increased ultimate strain, and complex strength relationships cited by the textile science literature.

The current study also supports a comparison between the textile science literature and twisted tendons. A statistically significant increase in minimum and maximum strain at 540° of rotation was observed in conjunction with a trend toward decreased maximum load and ultimate yield stress in load-to-failure testing. If these relationships are real, then in a theoretical model, increasing rotation of the BPTB graft in ACL reconstruction beyond 180° could have negative implications on the biomechanical properties of the implant. On the other hand, rotation up to 180° may actually strengthen the graft construct.

In conclusion, in a porcine model, cyclic loading of rotated BPTB constructs to 540° predictably shortened the length of the construct at the expense of increased strain. Although theoretically providing a technically easy solution for graft–tunnel length mismatch, the impact of increased strain on knee laxity has yet to be determined. Further clinical and basic science evaluation is required to determine the effects of physiologic loading of BPTB constructs in vivo and to evaluate the presence of this increased strain.

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