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Twisting and Braiding Reduces the Tensile Strength and Stiffness of Human Hamstring Tendon Grafts Used for Anterior Cruciate Ligament Reconstruction*

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Background: Twisting and braiding of four-strand hamstring tendon grafts used for anterior cruciate ligament reconstruction has been proposed, but not proven, as a method of improving tensile properties.
Hypothesis: Twisting and braiding four-strand human hamstring tendon grafts will have no significant effect on initial graft strength or stiffness.
Study Design: Paired in vitro biomechanical study.
Methods: In 12 matched cadaveric pairs, a doubled gracilis and semitendinosus tendon graft from one knee was twisted 180° over a 30-mm length, while the doubled tendon graft from the contralateral knee was prepared for biomechanical testing with the graft strands in a parallel orientation. For an additional 12 matched pairs, a doubled graft was braided into a weave while the contralateral graft was prepared for testing in a parallel orientation. All four strands of each doubled tendon graft were equally tensioned with weights before being clamped in a tendon-freezing grip. Tensile testing was then performed.
Results: Twisting decreased graft strength by 26% ($P < 0.01$) and stiffness by 43% ($P < 0.01$), while braiding reduced strength by 46% ($P < 0.01$) and stiffness by 54% ($P < 0.01$), compared with parallel-oriented grafts.
Conclusions: Equally tensioned, parallel four-strand human hamstring tendon grafts were significantly stronger and stiffer than twisted or braided four-strand hamstring tendon grafts.
Clinical Relevance: We caution against the use of twisted or braided four-strand hamstring tendon grafts for anterior cruciate ligament reconstruction.

Reconstruction of the ACL is the sixth most commonly performed orthopaedic surgical procedure in the United States.[34] Based on industry estimates, approximately 175,000 ACL reconstructions are performed annually in the United States.[34] The central-third patellar tendon graft and multiple-strand hamstring tendon autografts are the graft tissues most commonly used by knee surgeons to replace a torn ACL.[1,2,4,14,15,24] Because of the reported lower donor site morbidity, the use of hamstring tendon grafts has increased in recent years.[4,8,13,26,33] The initial tensile properties of single-strand hamstring tendon grafts are significantly lower than those of the normal ACL and central-third patellar tendon grafts,[7,11,17,20,32] but the properties of equally tensioned four-strand ham-

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string tendon grafts are significantly greater than those of the normal ACL and central-third patellar tendon grafts.[7,11,20] Because of the significant reduction in initial ACL graft strength that has been demonstrated to occur in animal models,[5,6,25] twisting or braiding of the multiple-strand hamstring tendon graft has been suggested as a possible method to further improve initial graft tensile properties.[10,27,29]

Biomechanical studies using animal tendons to determine the effects of braiding or twisting on initial graft strength and stiffness have not yielded clear conclusions. Braiding sheep digital extensor tendons significantly reduced strength (by 54%) and stiffness (by 85%) relative to parallel-strand controls.[19] However, braiding calf hoof extensor tendons had no effect on failure load but did reduce stiffness by about 30%, whereas twisting the tendons had no significant effect on strength or stiffness.[22] To our knowledge, no biomechanical study has examined the effects of twisting and braiding on the initial tensile properties of human hamstring tendons. The objective of this biomechanical study was to determine the effect of graft orientation (twisting and braiding) on the initial tensile properties of doubled gracilis and semitendinosus human hamstring tendon grafts.

MATERIALS AND METHODS

Specimen Preparation

Gracilis and semitendinosus free tendon grafts were harvested in an open fashion from 24 matched pairs of unembalmed human cadaveric knees (mean age, 63 ± 16 years; range, 29 to 94) obtained from the Department of Pathology at the Charité/Campus Virchow-Klinikum in Berlin, Germany. After harvest, the grafts were wrapped in gauze soaked with normal saline and frozen to –20°C. Before preparation and testing, the tendons were thawed overnight at room temperature. A large, blunt curette was used to remove all extraneous muscle and soft tissue from each tendon. The free ends of each tendon were whipstitched by using five throws of a No. 2 Ticron braided polyester suture (Sherwood, Davis & Geck, St. Louis, Missouri). The tendons were then wrapped in saline-soaked gauze, packaged in airtight plastic bags, and refrigerated at 4°C until testing the following day. Each tendon was allowed to warm to room temperature before biomechanical testing. Normal saline was used to keep the specimens moist during all phases of preparation and testing.

The length of each tendon was measured with a millimeter ruler. A single investigator (DHK) measured the cross-sectional area of each tendon with an area micrometer. A compressive load of 0.12 MPa was applied to each tendon for 2 minutes before recording the measurement.[11,20] Four cross-sectional area measurements were taken along the length of each tendon. Measurements were made at a distance of 30 and 60 mm from the center point of each tendon in both the proximal and distal directions. The mean cross-sectional area for the proximal and distal half of each tendon was calculated separately. The mean proximal and distal cross-sectional area measurements for matched gracilis and semitendinosus tendon sets were summed to produce the total cross-sectional area of the four-strand grafts.

One set of tendons from each matched knee pair was randomly chosen to be tested in a parallel orientation, and the other matched pair was tested in either a twisted or braided orientation. Grafts in the twisted group were rotated 180° over 30 mm (Fig. 1A) after being mounting in the specially designed cryogrips described later. Grafts in the braided group were fashioned by a "cross-and-twist" technique to braid the four tendon strands into a weave (Fig. 1B). Before biomechanical testing, the cross-sectional area of the twisted and braided tendon grafts was measured again at points 30 and 60 mm from the apex of the folded graft construct by using the area micrometer as described earlier. The length of the doubled gracilis and semitendinosus grafts was measured before and after twisting and braiding with a millimeter ruler.

Tensile Testing

The tendon constructs were tested to failure in tension on a servohydraulic materials testing system (Model 8521, Instron Corp., Canton, Massachusetts). To minimize soft tissue slippage or failure at the tendon-grip interface, we used two specially designed sets of tendon-freezing grips. For testing twisted and (contralateral) parallel doubled gracilis and semitendinosus grafts, a new design of tendon-freezing grips was employed (Fig. 2A). The new grip design maintained a circular configuration of the four

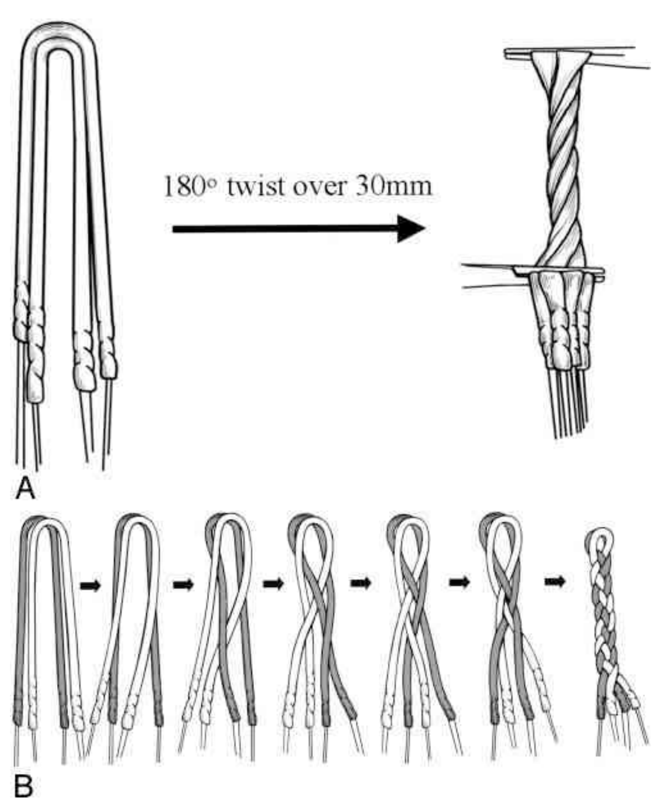

Figure 1. A, twisting technique; B, braiding technique.

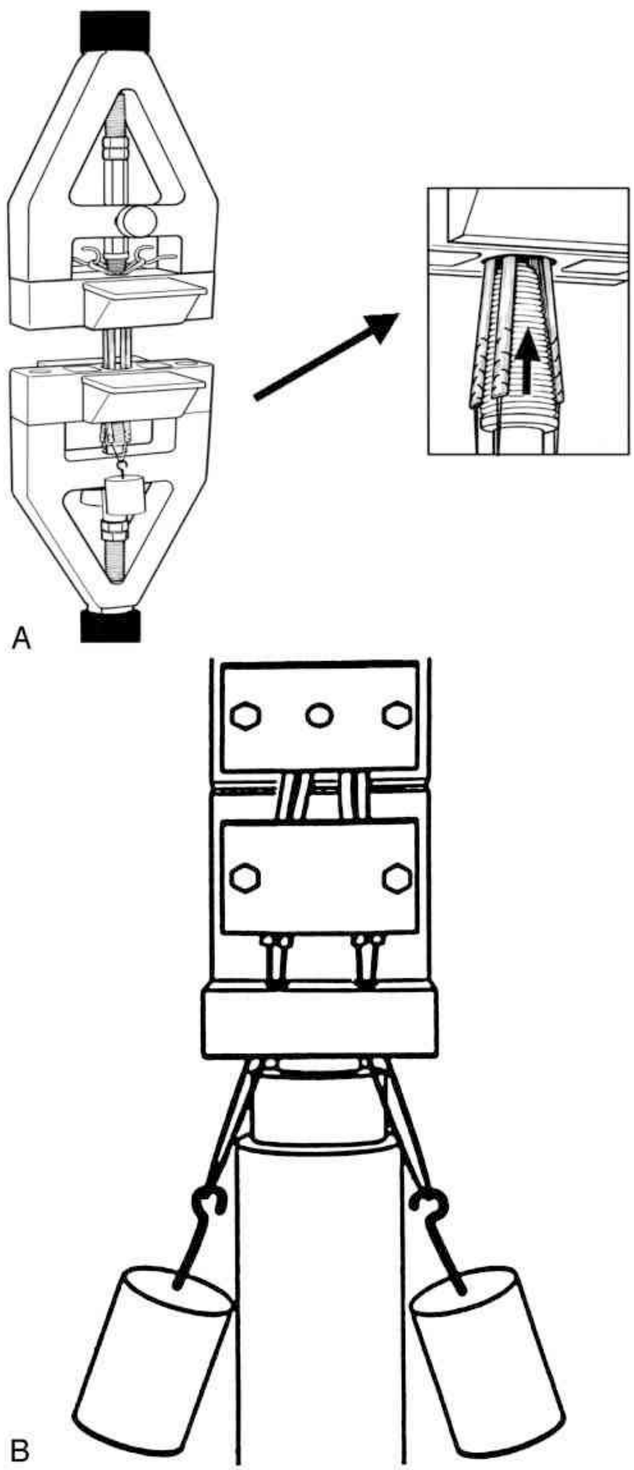

Figure 2. A, at left, the cryogrip system used in the twisting and parallel tendon testing group. This shows four hamstring tendons tested in a parallel orientation and equally tensioned with weights. At right is a close-up illustration of the clamping system, showing fixation of tendons into the bottom clamp. B, the cryogrip system used for the braided and parallel tendon testing group. This shows a parallel doubled gracilis and semitendinosus tendon graft placed in the grips and tensioned with hanging weights.

strands of hamstring tendons in the clamp as the proximal and distal ends were frozen by using dry ice held next to the jaws. This new grip design more accurately simulates the configuration of four-strand hamstring tendon grafts in vivo by decreasing flattening of the tendons when clamped. By decreasing tendon flattening at the grip interface, we were able to minimize off-axis loading, which is amplified when the grips are twisted 180°. To approximate the intraarticular length of the human ACL, we used a 30-mm grip-to-grip distance for testing. The sutures from the gracilis and semitendinosus tendon of each end of the graft construct were tied together, creating a loop, and equal tension was applied to all four strands of each graft construct by hanging a 500-g weight from the suture loops on each end of the graft. Grafts selected for testing in the twisted orientation were twisted by rotating the upper grip 180° relative to the fixed lower grip. This was done while maintaining the 75-N preload. The change in graft length was measured by recording the position of the upper clamp with the servohydraulic testing machine's linear variable differential transducer before and after twisting.

For testing braided and (contralateral) parallel doubled gracilis and semitendinosus tendon grafts, tendon-freezing grips similar in design to those described by Hamner et al.[11] were used (Fig. 2B). The tendon-freezing grips consisted of two flat plates with serrated teeth. The base plate was designed with a small chamber for dry ice to be placed within the grip.[21,23] Tendons were looped over the post in the upper tendon-freezing grip and the free ends of the tendons were equalized in length. The sutures from the gracilis and semitendinosus tendons on each end of the graft construct were tied together, creating a loop, and a 500-g weight was hung from the suture loop on each end of the grafts. This weight was maintained until the lower grip was completely clamped. A grip-to-grip distance of 30 mm was maintained. Dry ice was placed in the lower grip chamber and the clamp was tightened. A 75-N preload was applied to the graft construct, dry ice was placed in the upper grip chamber, and the upper clamp was tightened on the looped ends of the grafts. The 75-N preload was held constant as the grips were tightened to ensure that equal tension was maintained on all four tendon strands before testing.

The graft constructs were preconditioned by loading them cyclically from 50 to 100 N for 100 cycles at a frequency of 1 Hz. After preconditioning, the 75-N preload was reapplied to maintain graft tension, and the upper and lower grips were checked for tightness. The final grip-to-grip distance was measured with a handheld micrometer (Model CD-6CS, Mitutoyo Corp., Tokyo, Japan). A temperature probe (Model 8528–20, Cole-Parmer Instrument Company, Chicago, Illinois) was placed on the tendons at the midpoint between the two grips. When the temperature dropped below 13°C, the tendons were tested to failure at a strain rate of 100% elongation per second. Strain rate was determined from final measurement of the grip-to-grip distance after preconditioning.

The site and mode of failure were recorded for all tests. Failure load and stiffness were determined from load-

TABLE 1
Comparison of Characteristics of Twisted and Parallel Four-Strand Human Hamstring Tendon Grafts

Graft orientation	Area (mm^2)	Failure load (N)	Stiffness (N/mm)	Failure stress (N/mm^2)
Twisted (N = 12)	38.3 ± 7.3	2215 ± 759[a]	384 ± 96[a]	58.9 ± 19.1[b]
Parallel (N = 12)	39.3 ± 6.6	3000 ± 563	675 ± 143	78.0 ± 19.3

[a] Significantly lower value ($P < 0.01$).
[b] Significantly lower value ($P < 0.05$).

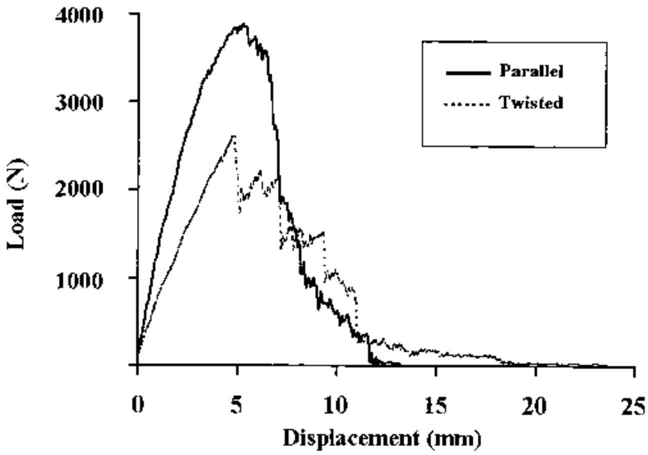

Figure 3. Typical load-displacement curves of a twisted doubled gracilis and semitendinosus tendon graft and parallel control

elongation curves by using data acquisition and analysis software (Series IX; Instron Corp.). Failure load was defined as the applied force at which the load-displacement curve deviated substantially from a straight line. Stiffness was defined as the slope of the linear region of the load-displacement curve. Failure stress was calculated by dividing the corresponding failure loads by the initial (unloaded) graft cross-sectional area.

Statistical Analysis

We tested the hypotheses that twisting or braiding has no significant effect on cross-sectional area, prepared graft length, failure load, stiffness, and failure stress. A paired Student's t-test was used to compare test data between the twisted or braided graft configurations and their respective parallel-strand controls. Significance was set at $P < 0.05$.

RESULTS

Twisting the tendons decreased the mean failure load by 26% ($P < 0.01$), mean graft stiffness by 43% ($P < 0.01$), and mean failure stress by 24% ($P < 0.05$) (Table 1). The mean cross-sectional areas of the twisted grafts before being twisted and that of the parallel controls were not statistically different. The cross-sectional area of the twisted tendons was not significantly different when compared with the sum of the areas of individual tendons before twisting. The load-displacement curves of the parallel controls typically displayed a sigmoid shape, with a single peak failure load and rapid failure (Fig. 3). On the other hand, the twisted tendons demonstrated a more gradual rise to a lower initial failure load and then displayed multiple peaks of subsequent lower failure loads in a step-wise fashion (Fig. 3).

Braiding the tendons decreased mean failure load by 46% ($P < 0.01$), mean graft stiffness by 54% ($P < 0.01$), and mean failure stress by 54% ($P < 0.01$) (Table 2). The mean cross-sectional areas of the braided grafts before being braided and that of the parallel controls were not significantly different. After braiding, the mean cross-sectional area of the braided hamstring tendon grafts increased by 21%, from 36.9 ± 10.2 mm^2 to 44.8 ± 10.8 mm^2 ($P < 0.05$), compared with their cross-sectional area in the unbraided state. Braiding also significantly decreased graft length by 15% from 115.4 ± 10.8 mm to 98.3 ± 10.1 mm ($P < 0.05$). The load-displacement curves showed different modes of failure (Fig. 4). The parallel controls exhibited the characteristic sigmoid shape with a single peak failure load and rapid failure, whereas the braided tendons initially failed at lower loads and then displayed sequential failures with multiple peaks similar to those of the twisted tendon group.

DISCUSSION

The purpose of this study was to determine the effect of twisting and braiding on the initial tensile properties of multiple-strand human hamstring tendon grafts. Our results indicate that twisting and braiding significantly reduced initial graft strength and stiffness compared with equally tensioned, parallel four-strand controls. Analysis of the load-displacement curves (Figs. 3 and 4) suggests that twisting and braiding reduced graft failure load and stiffness by producing unequal tension in the graft strands. Not only did ultimate failure occur at lower loads, there were also multiple peaks of stepwise failure before the complete tendon failure, with each peak likely representing disruption of individual hamstring tendon strands. Our data suggest that any multiple-strand tendon graft construct that results in unequal tensioning of the graft strands will result in a weaker and less stiff graft construct compared with grafts tested in an equally tensioned parallel orientation.

Our results for parallel four-strand tendon grafts compare favorably with those reported by Hamner et al.,[11] who examined human hamstring tendons using similar measurement and testing techniques. Although the mean failure loads (3090 ± 517 N and 3000 ± 563 N) and stiffness values (675 ± 143 and 715 ± 97 N/mm) for

TABLE 2
Comparison of Characteristics of Braided and Parallel Four-Strand Human Hamstring Tendon Grafts

Graft orientation	Area (mm^2)	Failure load (N)	Stiffness (N/mm)	Failure stress (N/mm^2)
Braided (N = 12)	44.8 ± 10.8	1673 ± 504[a]	330 ± 90[a]	37.1 ± 6.0[a]
Parallel (N = 12)	38.8 ± 8.7	3090 ± 517	715 ± 97	81.1 ± 10.3

[a] Significantly lower value ($P < 0.01$).

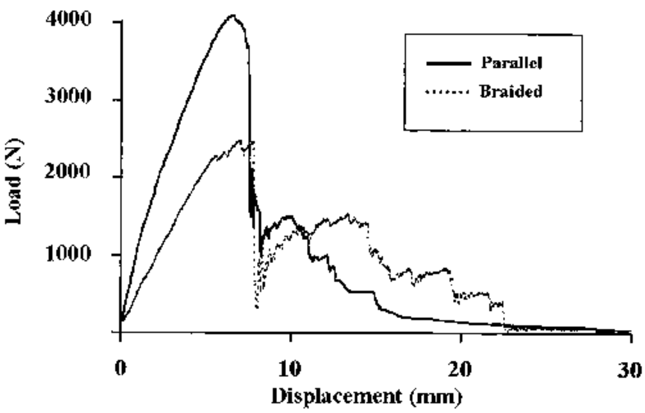

Figure 4. Typical load-displacement curves of a braided doubled gracilis and semitendinosus tendon graft and parallel control.

parallel grafts in the current study were lower than values reported by Hamner et al.[11] (4090 ± 295 N and 4590 ± 674 N, and 776 ± 204 and 861 ± 186 N/mm, respectively), the failure stress values (81.1 ± 10.3 and 78.0 ± 19.3 N/mm^2) in our study were similar to their results (77.7 ± 6.7 and 92.0 ± 18.7 N/mm^2). The difference in failure loads and stiffness, but similarity in failure stress, is most likely due to differences in tendon cross-sectional areas. The cross-sectional areas in the current study (38.8 ± 8.7 and 39.3 ± 6.6 mm^2) were lower than the cross-sectional areas reported by Hamner et al. (50.4 ± 3.5 and 52.9 ± 5.3 mm^2) and, as a result, lower failure loads and stiffness values would be expected.

Wilson et al.[31] compared the failure properties of twisted, double-looped human semitendinosus and gracilis tendon with that of 10-mm wide patellar tendon grafts. Although the failure loads for twisted hamstring tendons (2422 ± 538 N) compared favorably with those reported in the current study (2215 ± 759 N), stiffness values in the current study (384 ± 96 N/mm) were greater than values reported by Wilson et al. (238 ± 71 N/mm). Differences between stiffness values may be attributed to variations in testing protocols. Wilson et al. maximized the grip-to-grip distance in each test. In the current study, a standardized grip-to-grip distance of 30 mm was used. Wilson et al. also tested the grafts at a higher temperature, 21°C compared with 13°C in the current study, which may have affected graft stiffness.

Tis et al.,[30] in a recently published study, demonstrated that braiding caused a significant decrease in the strength and stiffness of human hamstring tendons. However, their values for failure load (427 ± 36 N for braided grafts and 532 ± 44 N for unbraided) and stiffness (76.1 ± 10 N/mm for braided and 139 ± 18 N/mm for unbraided) were much lower than those reported in the current study and in past investigations.[4,11,19,31] Because the authors did not report failure modes, we speculate that the large differences in failure properties could be attributed to their tissue-gripping methods, which may have resulted in grip-related soft tissue failures rather than midsubstance tendon failures. The authors attributed the differences to age-related changes in material properties. However, no other investigators have reported changes of this magnitude as being due to age of human hamstring tendon grafts (A. T. Hecker et al., unpublished data, 1997).

The results of the current study add to a largely inconsistent body of knowledge on the effects of braiding and twisting on various animal tendons. Nicklin et al.[19] reported that braiding sheep digital extensor tendons using two different techniques decreased graft strength by 47% and 57%, respectively, and reduced stiffness by 82% and 85%, respectively, compared with parallel strand controls. Although the reductions in graft strength are consistent with our findings in human specimens, the larger reductions in stiffness that they reported may have been observed because stiffness was measured during preconditioning cyclic loading. This is in contrast with our study, in which stiffness was measured after preconditioning, with the use of data from the load-elongation curve. Rittmeister et al.[22] reported that braiding calves' hoof extensor tendons reduced graft stiffness by 28%. However, twisting had no effect on stiffness and neither braiding nor twisting had any significant effect on failure load. Differences between the results of Rittmeister et al. and the current study may be explained by variation in testing techniques, twist configurations, and grip design. Rittmeister et al. did not use matched sets of tendons. Instead they compared three different strand configuration groups with a single control group of parallel tendons. In our study, we compared left-right sets of tendons from the same specimen, thus strengthening our statistical analysis. Rittmeister et al. also used a longer grip-to-grip length of tendon (55 mm) compared with the length used in the current study (30 mm), which may also have affected failure properties. Finally, only a small percentage of tendon grafts tested by Rittmeister et al.[22] failed at the tendon midsubstance (3 of 40).

Our results contradict the belief that twisting or braiding four-strand hamstring grafts improves initial graft tensile properties (Refs. 10, 29, 31; A. Perissinotto and A. Lelli, unpublished data, 2000). The practice of twisting and braiding multiple-strand hamstring grafts to increase mechanical properties has never been supported by experimental evidence. The perceived benefits of twisting and braiding hamstring grafts may have originated from previous studies examining the effect of twisting patellar tendon grafts on initial tensile properties. A 90° twist of human patellar tendon grafts reportedly resulted in a 30% increase in ultimate load and stress.[7] Although an investigation using a canine model showed that twisting yielded a similar increase in strength,[18] another recent study using human tissue found that twisting had no significant effect on initial graft strength and stiffness.[17] Furthermore, twisting of patellar tendon autografts has been shown to have no significant short-term effect on knee laxity, as determined by instrumented testing and clinical examination.[9]

Advocates of twisting or braiding tendon grafts believe that these techniques allow multiple-strand tendon grafts to act as a unit and behave like "rope."[10,27,29] However, when braiding is used in the textile industry in manufacturing yarns and ropes, it is for the purpose of improving the flexibility and handling characteristics of the material rather than for increasing tensile strength and stiffness.[12] Research on man-made fibers and yarns has shown that twisting is used in yarns to produce coherent structures having maximal flexibility and minimal resistance to bending stresses.[3,12,16] In fact, as the twist angle of fiber increases, its strength decreases.[12]

If rope or suture were unwound, the multiple stranded configuration would be harder to use but would demonstrate increased tensile properties, provided that all of the strands were placed under equal tension. When all strands of a multiple-strand construct are loaded equally, the corresponding strength is equivalent to the sum of the strengths of the individual strands. Therefore, to maximize the initial tensile properties of multiple-strand hamstring tendon grafts, equal tensioning of the graft strands should be emphasized. Hamner et al.[11] demonstrated that unequally tensioned four-strand human hamstring tendon grafts have lower failure loads (2831 $\pm$ 538 N compared with 4590 $\pm$ 674 N) and stiffness values (456 $\pm$ 97.7 N/mm compared with 861 $\pm$ 186 N/mm) compared with equally tensioned grafts. Twisting and braiding decreases the ability to obtain equal tension on the individual strands of a multiple-strand tendon graft and, therefore, reduces graft tensile properties.

Other disadvantages to braiding are the decrease in graft length and the increase in graft diameter, resulting in a shorter ACL graft that requires larger diameter bone tunnels. Larger bone tunnels and decreased graft length could potentially complicate graft fixation and decrease graft fixation options. Our study found that twisting the tendon had no significant effect on graft diameter but did decrease graft length by 4 mm.

One of the strengths of the current study is that we have attempted to simulate the geometry of the tibial and femoral bone tunnels by using a new tubular grip design. Therefore, our tests of twisted tendons more accurately simulate the loading of a twisted graft in the knee. Although we were unable to use the new grip system for tests of the braided tendons, we believe that the standard plate-style grips had minimal effect on failure properties because failure load and stiffness values for the two parallel control groups were not significantly different. This conclusion is further supported by the observation that the majority of hamstring tendon strands failed within the tendon midsubstance and not at the tendon-grip interface.

Another strength of our study is the use of paired sets of human tendons. Several other studies have used animal tendons; however, the results have been contradictory.[19,22] By testing human hamstring tendon grafts, we are able to present results that are applicable to the clinical setting. Also, because we tested paired sets of tendons, we were able to apply stronger statistical techniques in the analysis of our results than if we had tested unmatched specimens.

Extrapolation of the results of this study to clinical practice should be done with caution because of the limitations inherent in ex vivo biomechanical testing. Although unidirectional tensile testing to failure may simulate a hyperextension injury mechanism, it does not simulate the true loading conditions of the intact ACL. As the knee joint travels through its range of motion, the relative loading in the strands of the ACL and graft changes.[32] This effect could not be studied in our current model. Perhaps the application of torsional loads would better reflect the typical deceleration and multidirectional mechanism of ACL injury. It is also important to note that biomechanical testing has shown that the weakest areas of an ACL reconstruction at the time of initial surgery are at the points of fixation, especially on the tibial side.[28]

The results of this biomechanical study demonstrated that twisting or braiding four-strand hamstring tendon grafts significantly reduces initial graft strength and stiffness. On the basis of our results, we advocate caution against twisting or braiding hamstring tendon grafts to be used for ACL reconstruction surgery.

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