**In Vivo** Tensile Behavior of a Four-Bundle Hamstring Graft as a Replacement for the Anterior Cruciate Ligament

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**Summary:** The purpose of this study was to measure the in vivo tensile behavior of a double-looped semitendinosus and gracilis graft used to reconstruct a torn anterior cruciate ligament in the human knee. In 14 subjects: intraoperative tension was measured for each of the four graft bundles during passive motion from 0 to 90° of flexion. Two hypotheses were tested: (a) the peak tension carried by each of the four bundles was equal during passive motion, and (b) the mechanics of the bundles mimicked the functional bands of the native anterior cruciate ligament. The total tension was also calculated and used to determine strength requirements for fixation devices. The peak tensions of the four bundles during passive motion were not equal; however, enough tension was present in each bundle that load-sharing occurred between bundles. The pattern of tension between the anterior and posterior bundles mimicked the reciprocating load-sharing behavior of the functional bands of the native anterior cruciate ligament. Reciprocal tensile behavior was consistently achieved with the use of a single femoral tunnel centered on the most isometric line without the need for two separate femoral sockets. The maximum total tension was 296 N; this was nearly equal to the strength of one commonly used fixation device.

The double-looped semitendinosus and gracilis graft is an attractive alternative to the patellar tendon for replacing a torn anterior cruciate ligament. Although material testing results have shown that individual hamstring tendons are only 50% as strong as a 13.8 mm wide patellar tendon (15), a four-bundle graft, formed by looping semitendinosus and gracilis tendons over a fixation post (9), has an ultimate tensile strength of 4,108 N (5), which is 140% that of a 13.8 mm wide patellar tendon (2,900 N) and 240% that of the anterior cruciate ligament (1,710 N) (15). The double-looped graft also is attractive because the morbidity due to graft harvest is less than that of the patellar tendon (1).

Although the use of double-looped hamstring tendons as an anterior cruciate ligament graft has increased, little is known about the load-sharing properties between the bundles. Unequal load-sharing can predispose a single bundle to excessive loads, while stress-shielding other bundles (3). Therefore, the first goal of this research was to test the hypothesis that the peak tensions carried by each bundle in the double-looped hamstring graft were equal during passive flexion-extension.

Although large differences in bundle tensions are undesirable, small differences could be beneficial, by mimicking the functional bands of the native anterior cruciate ligament. Many studies have indicated that distinct regions of the anterior cruciate ligament provide knee stability at different flexion angles. For example, during passive motion of the leg, the posterolateral band tightens during extension whereas the anteromedial band tightens during flexion (2,8). Some authors have argued that single-bundle grafts, such as the patellar tendon and synthetics, do not provide the important reciprocating tension and slackening properties associated with the anteromedial and posterolateral bundles in the native anterior cruciate ligament (2,18). Thus, a second goal of this research was to test the hypothesis that at different flexion angles, the mechanics of a four-bundle graft mimic the functional bands of the native anterior cruciate ligament.

Concomitant with the increased use of double-looped hamstring grafts has been an increase in the number of fixation devices; however, the ultimate strengths of these devices vary widely. For example, a graft tied to an anchor with a Dacron tape supports a load of only 312 N, whereas a graft looped around a post supports a load of as much as 1,120 N (19). Because these strengths are all less than that of the graft for even a single tendon, the fixation device represents the weak link until biological fixation is achieved. To determine the strength requirement for fixation devices, a final objective...
or two knots in each suture. The length of the prepared tendon averaged 5 cm. The two limbs of a loop were identified by tying either one end of the suture. A relatively stiff (see error analysis later in this section) polyester, braided suture (no. 5 Ticron; Davis and Geck, Danbury, CT, U.S.A.) was sewn to the terminal 4 cm of each tendon by a whipstitch. The two limbs of a loop were identified by tying either one end of the suture. The length of the prepared tendon averaged 5 cm.

The semitendinosus and gracilis tendons were harvested with a tendon stripper, and the muscle was removed. The broader semitendinosus tendon was positioned lateral to the gracilis tendon, and the grafts were pulled, in parallel, up through the tunnels, looped from posterior to anterior around the post, and pulled down through the tunnels. Rotation was prevented by advancing the grafts in parallel from posterior to anterior around the post. The position of the femoral tunnel was chosen by placing a femoral guide through the tibial tunnel (size-specific femoral guide; Arthrotek). The femoral guide had a tongue-like extension that was hooked posterior or proximal to the intercondylar roof in the over-the-top position. This extension centered the femoral tunnel 5-7 mm distal to the proximal edge of the intercondylar roof at approximately the 11 o’clock position for the right knee or at 1 o’clock for the left knee. A 25 mm long, closed-end femoral tunnel was drilled.

The femoral fixation device consisted of a two-part screw (bone mulch screw; Arthrotek) (Fig. 1). The beam that extends from the body of the screw functioned as a post around which the tendons were looped. The screw was introduced through a tunnel drilled through the lateral femoral condyle.

The orientation of the four bundles with respect to each other and within the tunnels and intercondylar notch, was maintained by controlled passage of the graft under direct and arthroscopic visualization. The semitendinosus tendon was positioned lateral to the gracilis tendon, and the grafts were pulled, in parallel, up through the tunnels, looped from posterior to anterior around the post, and pulled down through the tunnels. Rotation was prevented by advancing the grafts in parallel from posterior to anterior around the post. Maintenance of the medial-lateral relationship after passage was verified by sliding each tendon around the post and checking its relative position.

The instrumentation system was secured to the tibia. A frame, supporting four load cells (Precision Measurements. Ann Arbor, MI, U.S.A.), was fixed to the proximal end of the tibia with two Kirschner wires drilled to achieve bicortical fixation (Fig. 2). Each bundle was attached to its respective load cell by passing the no. 5 polyester suture through the nipple-shaped end of the load cell and out the side where it was secured with a hose clamp (Fig. 3). Bench testing showed that each connection was capable of holding 120 N of tension without slipping.

The flexion angle of the knee was continuously measured by an electrogoniometer attached to the lateral side of the femur with a molded polyethylene cuff and Velcro (hook-and-loop) strap and to the tibia with a Velcro strap (Fig. 2). The 0° of flexion angle was established with the knee fully extended. The flexion angle for pretensioning the graft was chosen so that the bundles did not become slack during passive motion of the knee. To locate this angle, each bundle was initially pretensioned to 20 N at 30° of flexion and the knee was passively cycled three times. The flexion angle at which the graft tension was minimum was used to set the graft tension. Each bundle was tensioned to 5 N at this selected angle, which ranged from 25 to 40°. Because the length-tension patterns of grafts change during the initial cycles of passive flexion and extension (4), the knee was cycled 10 times from 0 to 90° of flexion to obtain a reproducible tension-flexion curve.

With use of a data acquisition system, the graft tensions in each of the four bundles and the flexion angle were simultaneously recorded at 100 Hz while the knee was passively cycled eight times.
from 0 to 90° by the surgeon. Because the operating table pre-vented full flexion of the knee, measurements could not be made past 90° of flexion. Variability in the tension of the graft during cycling was evaluated by comparing plots of the eight cycles from each subject. For each subject, the tension-flexion curve of the graft became reproducible by at least the fourth cycle; therefore, steady-state data from the repeatable eighth cycle were chosen for analysis.

To determine if the peak tensions carried by each bundle in the double-looped hamstring graft were equal, the bundle with the largest tension was compared with the bundle with the smallest tension for each subject through a paired t test analysis. A significant difference in peak tension (p < 0.05) indicated that the tensions between bundles were not equal.

To determine if the bundles of the graft mimicked the functional bands of the native anterior cruciate ligament, two separate two-factor repeated measures analyses of variance were utilized initially. In the first analysis, the summed tension in the two anterior bundles was compared with the summed tension in the two posterior bundles. In the second analysis, the summed tension in the medially positioned bundles was compared with that in the laterally positioned bundles. The two factors were bundle groups (two levels) and flexion angles (six levels). Because the initial analysis of the anterior and posterior bundle groups detected no significant difference in tension between the two groups when all flexion angles between 0 and 90° were included but did detect a strong effect of interaction between the tension in the bundle groups and the flexion angle of the knee (p < 0.001) all further statistical analyses for these bundle groups excluded flexion angle as a factor and instead consisted of separate analyses at 0 and 90°.

Three sources of error were analyzed, including friction between graft bundles and between the graft and tibial tunnel, compliance of the suture, and slippage of the suture sewn to the tendon. To determine if friction between graft bundles and between the graft and tibial tunnel caused graft tension inside the intercondylar notch to be underestimated, a pilot study was performed on a cadaveric knee prior to the in vivo experiments. With the use of procedures
similar to those described for the in vivo experiments, the graft tension in individual bundles was measured first with a regularly sized tibial tunnel and then with the tibial tunnel over-drilled so that the graft had a loose fit. Because there was no noticeable change in tension carried by the individual bundles, friction caused by the bundles contacting either each other or the tibial tunnel was negligible.

The second source of error was related to the compliance of the suture that bridged each bundle to the load cell. The tension in each bundle would be diminished if the stiffness of the suture connection was less than that of the tendon. To stiffen the connection, the length of the suture was limited to 7-10 mm, producing a calculated stiffness of 634 N/mm for a length of 7 mm and 444 N/mm for a length of 10 mm (6). The stiffness of each bundle was calculated to be 50 N/mm for a tendon that was 14 cm long (15). Error calculations were made by modeling the stiffness of the suture and bundle in series with one another. With this model, the suture connections reduced the tension in the graft by 7.3% for a 7 mm long suture bridge and 10.2% for a 10 mm long suture bridge. Therefore, the stiff suture attachment held errors in graft tension to less than 11%, whereas the greatest possible disparity between individual bundles was less than 3%. The estimates of error are conservative, because other compliant structures and junctions exist in the hamstring construct (e.g., femoral fixation post to bone and the bending stiffness of the fixation post), which, if included in the model, would reduce the actual error measurements to less than the calculated 11%. Moreover, because the suture length used in each connection was approximately the same for all subjects, the measurement errors were systematic and therefore do not affect the interpretation of the results.

The third possible source of error was lengthening of the graft-suture composite caused by either cinching, slippage, or tearing of the sutures through the tendon under load. This error was avoided by preconditioning the graft-suture composite at a pretension of 20 N per bundle, which was four times greater than the pretension of 5 N per bundle that was used during the actual measurement. Cinching, slippage, or tearing should have occurred under the higher tensions produced by the 20 N pretension and would not be expected to progress under the lower tension during the actual measurement. A repeatable tension-flexion curve observed in every knee by the fourth cycle provided evidence that slippage had stabilized. Because data were analyzed from the repeatable eighth cycle, it is unlikely

**FIG. 4.** Total graft tension versus flexion angle for nine subjects, with maximum tension values at full extension. Different lines were used for different subjects.

**FIG. 5.** Total graft tension versus flexion angle for five subjects, with maximum tension values at 90° of flexion. Different lines were used for different subjects.
that the preliminary “settling-in” of the graft affected the results.

RESULTS

The peak total tension in the four-bundle graft averaged 142 ± 59 N, with a range of 78-296 N for all 14 subjects. The peak tension occurred at full extension for nine subjects (Fig. 4) and at approximately 90° of flexion for five subjects (Fig. 5). The difference between the curves was correlated neither to any subject factors, including height, weight, gender, and age, nor to any intraoperative parameters, including graft diameter, the condition of the menisci, and the condition of the collateral ligaments.

The results relevant to the first hypothesis showed that, on average, the bundle with the largest peak tension was significantly different from the bundle with the smallest peak tension (p < 0.001): this indicated that the peak tensions in individual bundles were not the same. The maximum difference in tension between the two bundles averaged 23 ± 10 N, with the bundle with the largest peak tension averaging 54 ± 11 N and the bundle with the smallest peak tension averaging 30 ± 10 N. The average ratio of the bundle with the smallest peak tension to the bundle with the largest peak tension was 55 ± 15%, The smallest ratio for any subject was 37%. For all subjects, the bundle with the largest peak tension contributed 40 ± 14% of the peak total tension in the four-bundle graft.

The analyses used to address the second hypothesis indicated that there were no statistical differences between the summed tension in the medial and lateral bundles. However, there were statistically significant differences between the summed tension in the anterior and posterior bundles at 0° (p < 0.001) and 90° (p < 0.001) (Fig. 6). At full extension, the posterior bundles shared the majority of the load with an average of 58 ± 7%, while the anterior bundles had a significantly smaller contribution with only 42 ± 7% of the total tension. The opposite relationship was noted at 90° of flexion, with the anterior bundles carrying 65 ± 10% of the load, while the posterior bundles carried 35 ± 10%. This reciprocating relationship was exhibited in 13 of the 14 subjects at 0 and 90°.

DISCUSSION

Because little is known about the load-sharing behavior of the double-looped semitendinosus and gracilis graft as a replacement for a torn anterior cruciate ligament, one purpose of this study was to analyze the in vivo mechanics of this graft to determine if any of the bundles are stress-shielded. Although the peak tensions of the four bundles were not equal during passive motion, enough tension was present in each bundle that load-sharing occurred between them. The case with the most severe tension imbalance demonstrated that the bundle with the smallest peak tension developed 37% of the tension of the bundle with the largest peak tension. Because the specific amounts of tension necessary to maintain tissue integrity are unknown for biological grafts, it is difficult to place this percentage into a clinically meaningful perspective. Nevertheless, the results indicate that all bundles do carry some degree of tension during passive motion.

A second purpose of this study was to determine if the bundles mimic the reciprocal behavior of the functional bands of the native anterior cruciate ligament. Reciprocal behavior was observed between the anterior and posterior bundles of the double-looped semi-tendinosus and gracilis graft, indicating that friction existed in the femoral tunnel. Friction between the bundles and either the femoral tunnel or the fixation post prevented the tendon from acting like a rope over a frictionless pulley. The surgical procedure advocated making a tight fit between the graft and femoral tunnel to increase the rate of

FIG. 6. Typical tension curve showing reciprocal behavior of the tension in the anterior and posterior bundles.
biological incorporation. The 3 mm diameter post competed with the tendon for space in the 8-9 mm diameter femoral tunnel. As expected, resistance was always felt as the tendons were advanced around the post; this indicated friction in the femoral tunnel.

The observed reciprocal tensile behavior of the anterior and posterior bundles is evidence that the femoral tunnel was consistently positioned. A too posterior placement would cause the tension in all four bundles to increase in extension but not in flexion. A too anterior placement would cause the tension in all four bundles to increase in flexion but not in extension. For reciprocal tensile behavior to have occurred, as it did in 13 of the 14 subjects, the femoral tunnel had to be positioned partially anterior and posterior to the most isometric line. Our study has proven that reciprocal tensile behavior can be achieved with use of a single femoral tunnel centered on the most isometric line and that two separate femoral sockets are not required for reciprocal tensile behavior of the graft.

Although the normal anterior cruciate ligament has anteromedial and posterolateral bands that exhibit physiologic reciprocal tensile behavior, the clinical benefits of reciprocal tensile behavior of a graft have not been proven. However, there is clinical evidence that a multiple-bundle graft, composed of double-looped semitendinosus and gracilis tendons, provides better stability at 2 years than a single-bundle patellar tendon graft (11).

Theoretically, wear could occur from concentration of force between the graft and post and from movement of the graft around the pulley: however, this has not been a problem clinically. Patients that were intensively rehabilitated without braces and returned to unrestricted activities 4 months after reconstruction had acceptable stability and function when the double-looped semitendinosus and gracilis graft was passed around a post within the tibial tunnel (11).

The reciprocal behavior of the graft at the time of implantation should persist and may increase after biological incorporation. Conversion from mechanical to biological fixation is well established by the eighth week after implantation from a bond that develops between the tendon and the tunnel wall (16). Biological fixation causes the post to lose its pulley function. Greater differences in tension may result between the anterior and posterior bundles because equilibration of tension between two limbs of a loop cannot occur.

The tensile behavior of the medial and lateral bundles did not differ significantly; hence, there is no compelling reason to consistently place the semitendinosus tendon lateral and the gracilis tendon medial within the tibial and femoral tunnels. Interchanging the positions of the two tendons should have no bearing on the tensile behavior of the graft.

To determine whether currently available reconstruction methods are strong enough to maintain fixation during passive motion, the strength of fixation was compared with the total tension in the graft. In making this comparison, ideally the total tension in the graft would be measured for a pretension that restores normal anterior-posterior laxity because the pretension affects both laxity and total tension. Unfortunately, it was not possible to confirm that the normal laxity of the knee was restored with the 5 N pretension because the load frame, which was pinned to the tibia, prevented the application of an arthrometer.

Review of the method of pretensioning and comparison of it with other studies suggest that peak total tension in the double-looped hamstring graft was within the physiologic range. A 5 N pretension was chosen because it was the minimum tension that could be reliably maintained. The tension was applied to each bundle at the nadir of the graft tension-flexion curve, which occurred between 20 and 30°. This protocol prevented each bundle from becoming slack and allowed tension to be measured in each bundle at every flexion angle. The choice of pretensioning with 5 N per bundle, or a total of 20 N for the graft, was realistic as it is within the 11-66 N of pretension applied at 30° that was required to restore normal anterior-posterior laxity with use of a patellar tendon graft (14) and within the 8-31 N of pretension applied at 30° that was required to restore normal anterior-posterior laxity with use of an Achilles tendon graft (7). Furthermore, the range of maximum total tension of the double-looped hamstring graft (78-296 N) was similar to that of the normal anterior cruciate ligament (50-240 N) during passive motion (13).

Inasmuch as the pretension value was reasonable, the maximum total tension can be used to assess strength requirements of fixation devices during passive flexion. At the time of implantation, the fixation interface is the weakest link in the tendon-fixation construct. The maximum total tension was 296 N during passive motion. Comparison of this value with the ultimate strength of commonly used fixation methods—such as tying the graft to a fixation post with a suture (573 N) (17), tying the graft to either a button (430 N) or an anchor (312 N) with Dacron tape (19), looping the graft around a post (1,120 N) (19), compressing the graft between a bone and screw and soft-tissue washer (821 N) (17), and using an interference screw to fix a composite formed by wrapping the tendon around a bone block (354 N) (12)—indicates that the maximum total was nearly equal to the strength of at least one of these devices. Thus, the selection of the fixation device from the available possibilities should be made with caution.

It is unclear what caused the increase in total graft tension with flexion in some of the knees (Fig. 5). A possible explanation is the inconsistent placement of the femoral tunnel by which anterior placement could have resulted in an increase in tension with flexion. However, the use of the femoral guide prevented placement of the femoral tunnel anterior to the most isometric line. Moreover, the graft tension during pas-
sive motion is not affected by the anterior placement * of the tibial tunnel (7). Thus, it is unlikely that anterior placement of the femoral tunnel was the explanation for the increase in tension with flexion.

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