Hamstring Tendon Grafts for Reconstruction of the Anterior Cruciate Ligament: Biomechanical Evaluation of the Use of Multiple Strands and Tensioning Techniques*

BY DYSON L. HAMNER, M.D.†, CHARLES H. BROWN, JR., M.D.‡, MARK E. STEINER, M.D.§, AARON T. HECKER, M.S.#, AND WILSON C. HAYES, PH.D.#, BOSTON, MASSACHUSETTS

Investigation performed at the Orthopaedic Biomechanics Laboratory, Department of Orthopaedic Surgery, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston

Abstract

Background: **Our hypothesis that multiple, equally tensioned strands of hamstring graft used for reconstruction of the anterior cruciate ligament are stronger and stiffer than ten-millimeter patellar ligament grafts was tested biomechanically with use of tendons from cadavera.**

Methods: **In the first part of the study, we measured the strength and stiffness of one, two, and four-strand hamstring grafts, from fresh-frozen cadaveric knees, that had been tensioned equally when clamped. In the second part of the study, we compared four-strand grafts to which tension had been applied by hand and then clamped with similar grafts to which tension had been applied with weights and then clamped. The grafts for the two experiments were obtained from thirty-four paired and ten unpaired knees. We also studied the effects of cooling on the biomechanical properties of grafts by comparing patellar ligament grafts tested at 13 degrees Celsius with those tested at room temperature.**

Results: **Two equally tensioned gracilis strands had 185 percent of the strength and 210 percent of the stiffness (1550 ± 428 newtons and 336 ± 141 newtons per millimeter, respectively) of one gracilis strand (837 ± 138 newtons and 160 ± 44 newtons per millimeter, respectively). Two equally tensioned semitendinosus strands had 220 percent of the strength and 220 percent of the stiffness (2330 ± 452 newtons and 469 ± 185 newtons per millimeter, respectively) of one semitendinosus strand (1060 ± 227 newtons and 213 ± 44 newtons per millimeter, respectively).**

Four combined strands (two gracilis strands and two semitendinosus strands) that were equally tensioned with weights and clamped had the additive tensile properties of the individual strands. With the numbers available, four combined strands that were manually tensioned and clamped were not found to be significantly stronger or stiffer than two semitendinosus strands that were equally tensioned with weights (p > 0.07).

Conclusions: **Four combined strands that were equally tensioned with weights and clamped were stronger and stiffer than all ten-millimeter patellar ligament grafts that have been described in previous reports. All strands of a hamstring graft must be equally tensioned for the composite to have its optimum biomechanical properties.**

Clinical Relevance: **Because of the well recognized donor-site morbidity associated with the use of patellar ligament grafts for reconstruction of the anterior cruciate ligament, multiple-strand hamstring-tendon grafts have become an increasingly popular choice. Our data demonstrate that equally tensioned four-strand hamstring-tendon grafts have initial tensile properties that are higher than those reported for ten-millimeter patellar-ligament grafts; thus, from a biomechanical point of view, they seem to be a reasonable alternative.**

The functional importance of the anterior cruciate ligament is now universally accepted, and reconstruction of a torn ligament is one of the most commonly performed procedures[8]. Among the critical factors in the success of such reconstructions are the biomechanical properties of the graft material[5,8,10,12,13,18,21,22,25]. The two most commonly used grafts are the central third of the patellar ligament and the hamstring (gracilis and semitendinosus) tendons[1,8,23].

The patellar ligament has been considered the ideal graft material for reconstruction of the anterior cruciate ligament. The popularity of the graft is said to be related to the high initial strength, the potential for bone-to-bone healing, and the predictable success in restoring the stability of the knee[1,3,12,13,23,25]. However, the morbidity that is associated with use of the patellar ligament as

*No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article. Funds were received in total or partial support of the research or clinical study presented in this article. The funding sources were the Harvard Community Health Plan Research Foundation and Aircast Corporation.

†Sharp Rees-Stealy Department of Orthopaedic Surgery, 2929 Health Center Drive, San Diego, California 92123.

‡Department of Orthopaedic Surgery, Brigham and Women's Hospital, 75 Francis Street, Boston, Massachusetts 02115.

§Department of Orthopaedic Surgery, New England Baptist Hospital and Sports Medicine Brookline, 830 Boylston Street, Chestnut Hill, Massachusetts 02167.

#Orthopaedic Biomechanics Laboratory, Department of Orthopaedic Surgery, Beth Israel Deaconess Medical Center and Harvard Medical School, 330 Brookline Avenue, Boston, Massachusetts 02215.

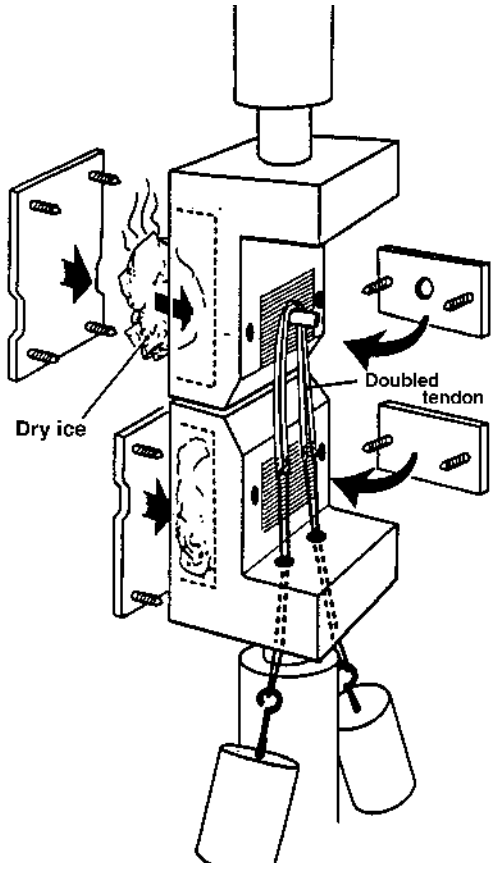

FIG. 1

Diagram showing the Cryo-Jaw clamp system with four combined strands placed under equally weighted (2.5-kilogram) tension. First, the lower chamber was filled with dry ice and the clamp was tightened. The four combined strands then were preconditioned and placed at a pretesting load of thirty-eight newtons. The upper chamber then was filled with dry ice, and that clamp was tightened before the composite graft was tested to failure.

graft material is also well recognized. The problems that are encountered include persistent pain at the donor site, chronic patellofemoral pain, quadriceps weakness, loss of motion, patellar fracture, and rupture of the patellar ligament[1,6,8,11,27,29].

On the basis of the results of tensile tests of single-strand hamstring grafts, investigators have estimated that two, three, or four strands of hamstring graft are necessary to reconstruct the anterior cruciate ligament[1,8,21,22,23,25]. To our knowledge, no study has evaluated the biomechanical properties of multiple-strand hamstring grafts for use in the repair of the anterior cruciate ligament.

Despite clinical studies that have shown successful results with use of hamstring grafts, there are concerns that such grafts may have considerable variability in strength and stiffness[1,8,20,21,23]. The present study was performed to address these unresolved issues.

Materials and Methods

Preparation of Specimens

Fresh-frozen knee specimens from cadavera were obtained through the Anatomical Gifts Program of Harvard Medical School. Knees that had evidence of previous operative treatment were excluded. The gracilis and semitendinosus tendons were removed after the knees had been thawed overnight at room temperature. The tendons were sectioned 180 millimeters from the tibial insertion and then sharply detached from the insertion. To prevent desiccation, individual tendons were wrapped in a towel moistened with saline solution, covered with aluminum foil, and placed in an airtight plastic bag. The tendons were immediately frozen at –20 degrees Celsius and used after a mean of three days. Before testing, the tendons were thawed overnight at 4 degrees Celsius and allowed to warm to room temperature on the day of testing. Normal saline solution was used to keep the specimens moist during all phases of preparation and testing.

The cross-sectional area of each tendon was measured, with use of an area micrometer, under a compressive load of 0.12 megapascal applied for two minutes[25]. These measurements were made at points thirty, sixty, 120, and 150 millimeters proximal to the tibial end of the tendon. The mean cross-sectional area of the distal half of the tendon was calculated as the mean of the measurements made at thirty and sixty millimeters, and the mean cross-sectional area of the proximal half of the tendon was the mean of the measurements made at 120 and 150 millimeters. The cross-sectional areas of the two and four-strand grafts were calculated by adding the cross-sectional areas of the individual strands.

Tensile Testing

A servohydraulic materials testing machine (model 1331; Instron, Canton, Massachusetts) was used for tensile testing. To avoid soft-tissue slippage or failure at the clamp-tendon interface, we used specially designed clamps that were a variation of the Cryo-Jaw[4,24,26]. A distance of thirty millimeters between the clamps was chosen to approximate the intra-articular length of the anterior cruciate ligament.

For tests of one and two strands, the axilla of a tendon (ninety millimeters from the tibial end of the graft) was placed over the post in the upper clamp (Fig. 1). The chamber containing the lower clamp was filled with dry ice, and the free ends of the tendon were secured by the serrated lower clamp. The tendon was able to slide over the post in the upper clamp when a tensile load was applied, which allowed equilibration of the tension between the two strands. Cyclical loading from thirty-eight to fifty newtons for ten cycles at one hertz was used to precondition the tendon; thereafter, a load of thirty-eight newtons was maintained. After the preconditioning, dry ice was placed in the chamber containing the upper clamp, which was then maximally

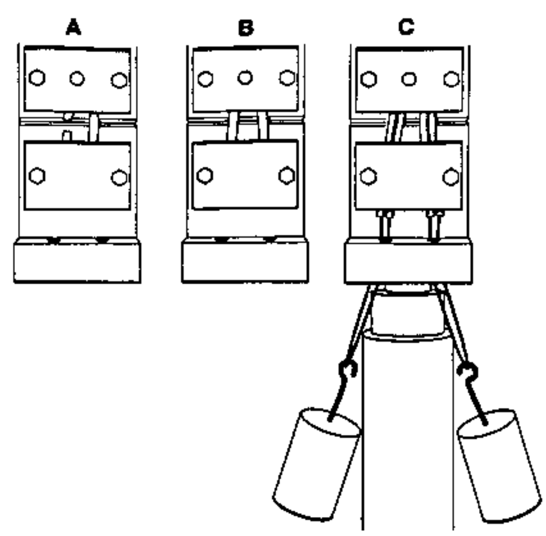

FIG. 2

Illustration depicting the testing configuration for one strand (*A*), two strands (*B*), and four combined strands (*C*) in the Cryo-Jaw clamp. The four combined strands are shown with equally applied weight.

TABLE I
COMPARISON OF ONE STRAND AND TWO STRANDS*

	Maximum Load (N)	Stiffness (N/mm)	Area (mm^2)	Maximum Stress (N/mm^2)
Gracilis tendon				
One strand (n = 12)	837 ± 138	160 ± 44	7.4 ± 1.1	113.1 ± 18.1
Two strands (n = 12)	1550 ± 428†	336 ± 141†	16.3 ± 3.0†	95.3 ± 19.1
Semitendinosus tendon				
One strand (n = 12)	1060 ± 227‡	213 ± 44‡	10.8 ± 2.2	99.3 ± 14.9
Two strands (n = 12)	2330 ± 452†‡	469 ± 185†‡	23.3 ± 3.6†	100.1 ± 12.9

*The values are given as the mean and the standard deviation.
†The value is significantly greater than that for one strand of the same tendon ($p < 0.001$).
‡The value is significantly greater than that for an equal number of gracilis strands ($p < 0.05$).

tightened on the looped end of the tendon. Thus, we had a model of two strands that were equally tensioned when clamped. If a single strand was to be tested, the segment from the proximal end of the tendon was cut at this point, leaving a single distal strand (Fig. 2).

In order to test four combined strands, a number-2 Ethibond suture (Ethicon, Somerville, New Jersey) was placed in the ends of the gracilis and semitendinosus tendons in a whipstitch fashion (four throws). The tendons were placed over the post in the upper clamp. In tests of four combined strands with manual tensioning, the free ends of the tendons were held by one person who grasped the sutures with two hands in an attempt to place equal tension on all strands. Dry ice was placed in the chamber containing the lower clamp, and the lower clamp was tightened by a second person as manually applied tension was maintained. After preconditioning, dry ice was placed in the chamber containing the upper clamp and that clamp was tightened on the looped ends of the tendons.

In tests of four combined strands with tension applied by weights, a 2.5-kilogram weight was attached by sutures to each end of the gracilis and semitendinosus tendons (Figs. 1 and 2). Dry ice was placed in the chamber containing the lower clamp, which then was tightened with each strand tensioned equally by the weights. After preconditioning, dry ice was placed in the chamber containing the upper clamp and that clamp then was tightened on the looped ends of the tendons.

After preconditioning and clamping, the clamp-to-clamp length was measured with handheld digital calipers (model CD-6P; Mitutoyo, Tokyo, Japan) while maintaining a load of thirty-eight newtons. Marks were made at each clamp-tendon interface with India ink to evaluate the tendons for possible slippage in the clamp, and two parallel marks were made at the mid-substance of the tendon to assist in the determination of the site and region of tendon failure. A temperature probe (model HH 70 TC; Omega Engineering, Stamford, Connecticut) was placed on the tendon or tendons at the midpoint between the two clamps[14]. When the temperature dropped to 13 degrees Celsius, the tendon or tendons were tested to failure at a strain rate of 100 percent elongation per second. One specimen was tested when the temperature of the mid-substance was 10 degrees Celsius.

The site and mode of failure were recorded for all tests. Maximum load and stiffness were determined from load-elongation curves with use of data-acquisition software (Labtech Notebook; Laboratory Technologies, Wilmington, Massachusetts). Maximum load was the peak load measured[30], stiffness was measured in the linear region of the deformation curve, and maximum stress was calculated by dividing the maximum load by the cross-sectional area.

Test Groups

Group I: Comparison of One Strand and Two Strands

Tendon specimens were obtained from twelve cadavera (twenty-four paired knees). The mean age was eighty years (range, seventy to 102 years) at the time of death. The tendons from one knee of a pair were used for the one-strand test, and the tendons from the contralateral knee were used for the two-strand test. The sides were alternated between cadavera. The two strands were equally tensioned when clamped.

Group II: Comparison of Two Strands and Four Combined Strands

Tendon specimens were obtained from five cadavera (ten paired knees). The mean age was fifty-three years (range, thirty-seven to eighty-eight years) at the time of death. The two and four-strand tests were performed on paired knees, as described for the one and two-strand tests. The two strands were equally tensioned when clamped, and the four combined strands were equally tensioned with weights when clamped.

Group III: Four Combined Strands

Tendon specimens were obtained from unpaired knees, and four combined strands (two gracilis strands and two semitendinosus strands) were tensioned manually or with weights and then clamped. Five knees from cadavera (mean age at the time of death, sixty years; range, forty-seven to eighty-three years) were used for the manual tests, and five other knees from cadavera (mean age at the time of death, eighty-two years; range, seventy-six to eighty-five years) were used for the tests with the weights.

Tests of Cooling Effect

To determine the effect of cooling on the biomechanical properties of tendon grafts, we performed a series of experiments comparing patellar ligament (bone-ligament-bone) specimens tested at room temperature with patellar ligament specimens tested at 13 degrees Celsius. Six fresh-frozen patellar ligament grafts were obtained from cadavera; five of the individuals had had a mean age of seventy-nine years (range, sixty-six to eighty-eight years) at the time of death, and the age of the sixth was unknown. Before mechanical testing, the specimens were thawed overnight at room temperature. Three-millimeter-wide grafts were cut, with a scalpel, from the medial and lateral sides of each patellar ligament specimen. Bone blocks measuring twenty millimeters wide, thirty millimeters long, and eight millimeters thick were obtained from the patella and the tibial tubercle with use of an oscillating saw (model 9004-210; Stryker, Kalamazoo, Michigan).

Slippage of the bone blocks in the clamps was minimized by use of a narrow tendon that decreased the potential maximum load to failure. Before mechanical testing, the cross-sectional area of each graft was measured with an area micrometer. Alternating between specimens, we assigned the medial and lateral grafts from the same patellar ligament to testing either at room temperature or at 13 degrees Celsius. A total of six medial and six lateral patellar ligament specimens were tested. The bone plugs were secured to the materials testing machine with the Cryo-Jaw clamp system. Dry ice was placed in the chambers when the tendons were to be tested at 13 degrees Celsius.

A temperature probe was used to measure the temperature of

TABLE II
COMPARISON OF TWO STRANDS AND FOUR COMBINED STRANDS*

	Maximum Load (N)	Stiffness (N/mm)	Area (mm^2)	Maximum Stress (N/mm^2)
Two gracilis strands (n = 5)	1550 ± 369	370 ± 108	20.5 ± 3.5	77.9 ± 25.6
Two semitendinosus strands (n = 5)	2640 ± 320	534 ± 76	30.6 ± 2.4	86.3 ± 10.0
Four combined strands (n = 5)	4090 ± 295†‡	776 ± 204†	52.9 ± 5.3†‡	77.7 ± 6.7

*Equal tension was applied with weights on each strand when clamped. The values are given as the mean and the standard deviation.
†The value is significantly greater than that for two gracilis strands ($p < 0.05$).
‡The value is significantly greater than that for two semitendinosus strands ($p < 0.05$).

the tendon at the midpoint between the clamps. Six specimens were tested when the midpoint of the tendon reached 13 degrees Celsius, and six specimens were tested with the tendon at room temperature. A fifty-newton preload was applied, and the clamp-to-clamp distance was measured with handheld digital calipers. The tendons were tested to failure at a strain rate of 100 percent elongation per second. The site and mode of failure were recorded for all tests. We used the same methods as those used for the hamstring grafts to determine the tensile parameters of the patellar ligaments.

Statistical Analysis

A two-way analysis of variance, with the number of tendon strands as the paired factor and the type of tendon as the grouping factor, was used to evaluate the effects of the number of strands and the type of tendon on maximum load, stiffness, cross-sectional area, and maximum stress. A Student t test was used to compare the two semitendinosus strands with the four combined strands. Linear regression was used to evaluate the relationship between maximum load and cross-sectional area. A Student t test was used to compare the maximum stress and stiffness of the patellar ligaments at 13 degrees Celsius with those at room temperature.

Results

Group I: Comparison of One Strand and Two Strands

The mean maximum load (and standard deviation) was 837 ± 138 newtons for the one-strand gracilis grafts, 1550 ± 428 newtons for the two-strand gracilis grafts, 1060 ± 227 newtons for the one-strand semitendinosus grafts, and 2330 ± 452 newtons for the two-strand semitendinosus grafts. Two strands of the same tendon had approximately twice the strength and stiffness as one strand (Table I). The two-strand gracilis grafts and the two-strand semitendinosus grafts had a mean of 185 and 220 percent, respectively, of the maximum load of the one-strand grafts.

The mean stiffness of the one-strand gracilis grafts was 160 ± 44 newtons per millimeter, and that of the two-strand grafts was 336 ± 141 newtons per millimeter. The mean stiffness of the one-strand semitendinosus grafts was 213 ± 44 newtons per millimeter, and that of the two-strand grafts was 469 ± 185 newtons per millimeter. The two-strand gracilis grafts and the two-strand semitendinosus grafts had a mean of 210 and 220 percent, respectively, of the stiffness of the one-strand grafts.

The average, cross-sectional area of the one-strand gracilis grafts was 7.4 ± 1.1 square millimeters and that of the two-strand grafts was 16.3 ± 3.0 square millimeters. The one-strand semitendinosus grafts had a mean area of 10.8 ± 2.2 square millimeters and the two-strand grafts, a mean area of 23.3 ± 3.6 square millimeters.

The mean maximum load ($p < 0.001$) and stiffness ($p = 0.02$) of the semitendinosus grafts were greater than those of the gracilis grafts. On the average, the semitendinosus grafts had 146 percent of the area, 127 percent of the strength, and 133 percent of the stiffness of the gracilis grafts. With the numbers available, we detected no significant difference in mean maximum stress with respect to either the type of graft or the number of strands.

Of the forty-eight grafts that were tested, thirty-four failed at their mid-substance, nine failed at the clamp-tendon interface, three slipped within the clamps, and two both tore at the clamp-tendon interface and slipped within the clamps.

Group II: Comparison of Two Strands and Four Combined Strands

The mean maximum load was 1550 ± 369 newtons for the two-strand gracilis grafts, 2640 ± 320 newtons for the two-strand semitendinosus grafts, and 4090 ± 295 newtons for the four-strand combined grafts (Table II). Four combined strands equally tensioned with weights when clamped had tensile properties that approximated the additive properties of the two-strand components. The mean maximum load for the four combined strands was 98 percent of the combined maximum loads for two gracilis and two semitendinosus strands.

The two-strand gracilis grafts had a mean stiffness of 370 ± 108 newtons per millimeter; the two-strand semitendinosus grafts, 534 ± 76 newtons per millimeter; and the four-strand combined grafts, 776 ± 204 newtons per millimeter. The stiffness of the four combined strands averaged 86 percent of the combined stiffness of the two gracilis strands and the two semitendinosus strands. With the numbers available, we detected no significant difference in mean maximum stress among the three types of grafts (two gracilis strands, two semitendinosus strands, and four combined strands). Of the fifteen composite grafts that were tested, fourteen failed at their mid-substance and one slipped within the clamps.

Group III: Four Combined Strands

The mean maximum load for the four-strand grafts was 2831 ± 538 newtons when the tension had been

TABLE III
COMPARISON OF FOUR COMBINED STRANDS TENSIONED MANUALLY AND FOUR COMBINED STRANDS TENSIONED WITH WEIGHTS*

Type of Tension	Maximum Load *(N)*	Stiffness *(N/mm)*	Area *(mm²)*	Maximum Stress *(N/mm²)*
Manual (n = 5)	2831 ± 538	456 ± 97.7	39.8 ± 4.6	71.6 ± 13.9
Weighted (n = 5)	4590 ± 674†	861 ± 186†	50.4 ± 3.5	92.0 ± 18.7†

*The values are given as the mean and the standard deviation.
†The value is greater than that for four combined strands tensioned manually when clamped ($p < 0.05$).

applied manually and 4590 ± 674 newtons when it had been applied with weights (Table III). The mean stiffness of the manually tensioned grafts measured 456 ± 97.7 newtons per millimeter, and that of the grafts tensioned with weights measured 861 ± 186 newtons per millimeter. The grafts with four strands equally tensioned with weights had a mean of 162 percent of the strength and 189 percent of the stiffness of those with the four manually tensioned strands.

The mean cross-sectional area of the grafts that had been tensioned with weights (50.4 ± 3.5 square millimeters) was greater than that of the grafts that had been tensioned manually (39.8 ± 4.6 square millimeters), and this difference inflated the maximum load and stiffness differential. The mean maximum stress was significantly greater in the grafts that had been tensioned with weights (92.0 ± 18.7 newtons per square millimeter) than in those that had been tensioned manually (71.6 ± 13.9 newtons per square millimeter) ($p < 0.05$).

With the numbers available, the four combined strands that had been tensioned manually were not found to be significantly stronger or stiffer than the two-strand semitendinosus grafts in groups I and II ($p > 0.07$). Three of the four-strand grafts that had been tensioned manually tore at their mid-substance, one tore at the clamp-tendon interface, and one slipped within the clamps. Four of the four-strand grafts tensioned with weights tore at their mid-substance, and one tore at the clamp-tendon interface.

Maximum Load and Cross-Sectional Area

There was a strong positive linear correlation between maximum load and cross-sectional area for one strand, two strands, and four combined strands tensioned with weights ($r^2 = 0.996$ for the mean data points) (Fig. 3). The four combined strands that had been manually tensioned demonstrated a lower load for a given cross-sectional area.

Tests of Cooling Effect

With the numbers available, we detected no significant difference between the patellar ligaments tested at 13 degrees Celsius and those tested at room temperature with respect to mean maximum stress ($p = 0.42$) or stiffness ($p = 0.11$). The sites of failure were similar for the two groups: five mid-substance tears,

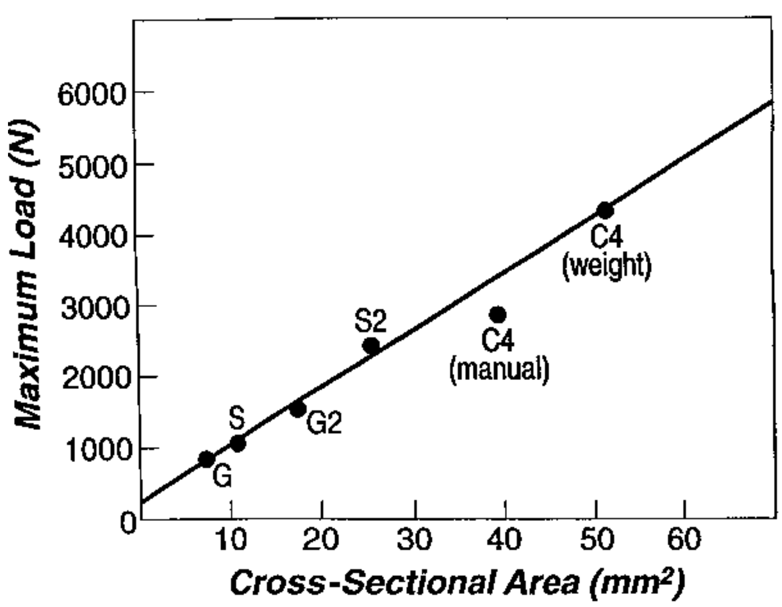

FIG. 3

Graph plotting the changes in maximum load in relation to the cross-sectional area for one gracilis strand (G), two gracilis strands (G2), one semitendinosus strand (S), two semitendinosus strands (S2), four combined strands that were manually tensioned (C4 [manual]), and four combined strands that were tensioned with identical weights (C4 [weight]). There was a strong positive linear correlation between maximum load and cross-sectional area for one strand, two strands, and four combined strands (tensioned with weights) ($r^2 = 0.996$ for the mean data points). Maximum load = 70.5 × (cross-sectional area) + 222.

five tears at the tibial insertion, and two tears at the patellar insertion.

Discussion

During the past decade, there has been an increased use of hamstring grafts with multiple strands to reconstruct the anterior cruciate ligament[1,8,20,21,23]. This trend is believed to be related to improved fixation techniques and the perception that hamstring grafts are associated with less morbidity than patellar ligament grafts[1,8,20,21,23,29,31]. Patellar ligament grafts continue to be popular because of their long record of providing stability; however, concerns associated with their use include pain at the donor site, chronic patellofemoral pain, loss of motion of the knee, quadriceps weakness, patellar fracture, and rupture of the patellar ligament[1,6,8,11,23,27,29].

Although many investigators have reported on reconstruction of the anterior cruciate ligament, to our knowledge there have been only two prospective studies comparing patellar ligament and four-strand hamstring tendons as graft sources[1,23]. However, in both of these studies, a more conservative rehabilitation program was used because of the weaker initial fixation of the hamstring grafts. The more conservative rehabilitation program may have biased the results in favor of the hamstring grafts. Marder et al.[23] prospectively compared the results of use of a four-strand hamstring graft with those of a patellar ligament graft in seventy-two patients who had a chronic tear of the anterior cruciate ligament. The patients in both groups were managed with limited motion and weight-bearing for the first six weeks. Pain in the anterior aspect of the knee was noted in seventeen (24 percent) of the seventy-two patients. No difference with respect to the type of graft was observed. A loss of extension of at least 5 degrees was observed in 11 percent (four) of the thirty-seven knees that had a patellar ligament graft and in only 3 percent (one) of the thirty-five knees that had a hamstring graft. Overall, the stability was similar in the two groups. Marder et al. suggested that delayed rehabilitation may have contributed to pain in the anterior part of the knee and loss of motion in both groups.

Aglietti et al.[1] also compared the results of anterior cruciate reconstruction with a four-strand hamstring graft with those of reconstruction with a patellar ligament graft, in sixty patients who had a chronic tear. The rehabilitation program included limited motion with the knee in a brace for four weeks followed by limited weight-bearing for eight weeks. Moderate patellar crepitus was noted in 17 percent (five) of the thirty knees that had a patellar ligament graft and in only 3 percent (one) of the thirty knees that had a hamstring graft. There was minimum loss of extension in both groups, but it was more common in the group that had a patellar ligament graft. Fifty percent (fifteen) of the thirty knees that had a patellar ligament graft had a flexion contracture of 5 degrees or less, whereas only 3 percent (one) of the thirty knees that had a hamstring graft had a similar loss of extension. Despite the problems of patellofemoral pain and limited motion, Aglietti et al. still concluded that the patellar ligament graft was preferable to the hamstring graft because the return of stability was more reliable. Their conclusion was based on data obtained from testing with a KT-2000 arthrometer, which demonstrated that 13 percent (four) of the thirty knees that had a patellar ligament graft had a side-to-side difference in anterior displacement of more than five millimeters at thirty pounds (133 newtons), whereas 20 percent (six) of the thirty knees that had a hamstring graft had such a difference. This difference appears to be insufficient to support the conclusion that a patellar ligament graft produces a more stable repair than a hamstring tendon graft.

Aglietti et al.[1] and Marder et al.[23] reported the results of isokinetic testing of knees after repair with use of a patellar ligament graft and after repair with use of a hamstring graft. The mean peak quadriceps torque at 60 degrees per second for the knees that had a patellar ligament graft and for those that had a hamstring graft was 88 and 91 percent of normal, respectively, in the study by Marder et al. and 91 and 89 percent of normal, respectively, in the study by Aglietti et al. The data appear to suggest that mild quadriceps weakness may be inherent to injuries and reconstructions of the anterior cruciate ligament rather than to the source of the graft. Quadriceps weakness and loss of motion have been minimized with currently used accelerated rehabilitation programs that focus on regaining motion and strength as rapidly and intensively as possible while protecting the repair. Weakness of the hamstring muscles has not been known to be a major problem when those tendons are used to provide graft material[8].

Fracture of the patella and rupture of the patellar ligament after removal of a section of the ligament for graft material are major problems; however, it appears that the prevalence of these problems is low[6,11]. Overall, clinical studies have suggested that there is minimum long-term morbidity with use of either graft source (hamstring or patellar ligament), but patients who have a hamstring graft appear to have less pain and a slightly easier course of rehabilitation in the early postoperative period[1,8,20,29].

We evaluated the tensile properties of one, two, and four-strand hamstring grafts that were secured by clamps. We were unable to find any study that evaluated the biomechanical properties of multiple-strand hamstring grafts. We determined that the biomechanical properties of hamstring grafts are proportional to the number of strands when each strand was tensioned equally during the clamping process. Four combined strands that were manually tensioned and held in a clamp had the strength and stiffness of only two semitendinosus strands that were equally tensioned.

Our clamping system was developed to obviate the problem of slippage at the clamp-tendon interface. To create a more rigid structure, the ends of the tendon were frozen with dry ice in special Cryo-Jaw clamps[4,24,26]. The tests were performed when the temperature of the midpoint of each graft reached 13 degrees Celsius. In order to determine the effect, if any, of cooling on the biomechanical properties of tendon grafts, we also compared patellar ligament grafts tested at room temperature with patellar ligament grafts tested at 13 degrees Celsius. Patellar ligament grafts were used because the large bone blocks at each end of these grafts made it possible to avoid slippage of the graft in the clamps at room temperature. The grafts were relatively small (three millimeters in diameter), and there were variations, among specimens, in cross-sectional area. To minimize the effect of this variation, maximum stress was calculated to evaluate strength. We detected no significant difference, with the numbers available, between the patellar ligament grafts tested at room temperature and those tested at 13 degrees Celsius with respect to mean maximum stress or stiffness.

The major advantage of the Cryo-Jaw clamp is that it allows low clamping pressures that only slightly distort the clamped tissues and thus there is less chance of failure at the clamp-tendon interface. The tensile strength and stiffness of a graft can be determined only if the specimen fails within the graft substance[2]. Any tearing at the insertion into the clamps may result in lower measurements for strength and stiffness. We occasionally observed slippage at the sites of insertion into the clamps; therefore, the results for maximum load, stiffness, and maximum stress may have been slightly underestimated.

The testing protocol developed for the present study allowed each strand of the two-strand grafts to be tensioned equally during the clamping process. Either the gracilis or the semitendinosus tendon was looped over the post in the upper clamp, and then the two free ends of the tendon were secured in the lower clamp. The tendon could freely slide over the upper post to equalize any uneven tension in the two strands as the graft was cycled ten times to a load of fifty newtons. After this equilibration process was completed, and a resting load of thirty-eight newtons was maintained, the upper clamp was tightened on the looped tendon. We believe that this procedure resulted in equal tension on each strand and in the subsequent failure test each strand was equally loaded.

The clamping process that was used for the two strands could not be employed for the four combined strands. When four combined strands were tested, the gracilis and semitendinosus tendons were looped over the post in the upper clamp and tension was maintained on the separate ends of the tendons as the lower clamp was tightened. Uneven loading would have occurred in the subsequent failure tests if the gracilis and semitendinosus strands had not been equally tensioned when the lower clamp was tightened. We compared the results of tension applied manually to the four combined strands during clamping with those of tension applied with weights. In the tests in which tension was applied manually, the four combined strands were held by one individual who attempted to apply an equal load to each strand. In the tests in which tension was applied with weights, a 2.5-kilogram weight was hung from each tendon during the clamping process. Our results demonstrated that tension applied by hand was not effective in equalizing the load between the gracilis and semitendinosus strands. The composite functioned much like two semitendinosus strands. Application of

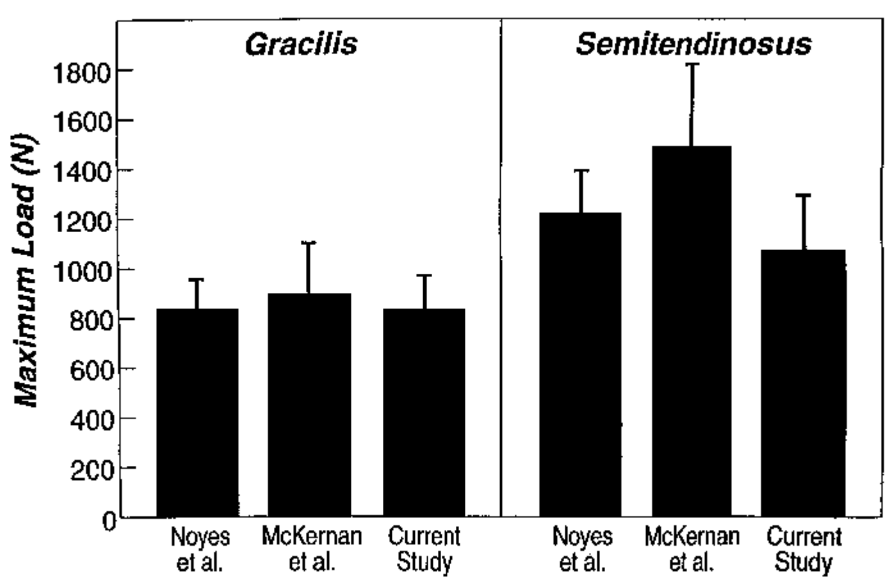

FIG. 4

Graph comparing the strength (maximum load) of one strand of gracilis tendon and that of one strand of semitendinosus tendon in the current study with those reported by McKernan et al.[22] and Noyes et al.[25]. The values are given as the mean and the standard deviation.

the tension with attachment of an equal (2.5-kilogram) weight to each strand during the clamping process resulted in the composite with the greatest strength and stiffness.

Noyes et al.[25] reported that a one-strand gracilis graft and a one-strand semitendinosus graft had 49 and 70 percent, respectively, of the strength of the normal anterior cruciate ligament in a young individual. On the basis of clinical investigations and studies of animals that have demonstrated loss of strength of the graft during healing, many surgeons have attempted to increase strength with use of four-strand hamstring grafts[3,7,8,12,15,16,19,21,25]. It has been suggested that the mechanical properties of graft strands are additive[8,23]. On the basis of the data reported by Noyes et al.[25], it has been estimated that four combined strands have 250 percent of the strength of the normal anterior cruciate ligament. In the present study, four combined strands equally tensioned with weights and then clamped had more than 250 percent of the strength of the normal ligament as described by Noyes et al.[25] (1725 ± 269 newtons). Woo et al.[32] reported a slightly higher value for the strength of the normal ligament (2160 ± 157 newtons).

Our results for the strength of one-strand gracilis grafts and one-strand semitendinosus grafts are comparable with those reported by Noyes et al.[25] and McKernan et al.[22] (Fig. 4). The slight variability among the results of the studies may be explained by variations in the size and the preparation of the specimens and the clamping technique. We tested specimens that were thirty millimeters long and had been obtained from the distal end of the tendon. The length and region of the tendons tested in the other studies were not identified.

It should be noted that we used specimens from the cadavera of older individuals. Two of us (A. T. H. and C. H. B., Jr.) and colleagues, who used the same test protocol as that employed in the present study, recently reported on the strength of hamstring grafts obtained from the cadavera of young donors. Those authors were unable to document any effect of age on the mechanical properties of hamstring tendons. Blevins et al.[5] reported that the age of the donor does not affect the strength of patellar ligament grafts. In contrast, Woo et al.[32] documented a decrease in the strength and stiffness of the anterior cruciate ligament with increasing age.

Caution should be used in extrapolating the results of our study to clinical estimates of the strength of hamstring grafts. At the time of reconstruction, the weakest points in an anterior cruciate ligament construct are its points of fixation[16,19,31]. We previously reported that the fixation strength of four combined strands in an anterior cruciate reconstruction of a cadaveric knee from an elderly individual was 821 newtons[31], a value that was considerably less than the values obtained in the present study, in which thirty-millimeter strands of tendon were clamped directly. In the first eight weeks after reconstruction, the graft heals within the bone tunnels and failure can subsequently occur in the mid-substance of the graft[16,19,28]. Studies of animal models have demonstrated considerable weakening of biological grafts compared with their initial strength[3,12,15,19].

Our laboratory study demonstrated that a combined four-strand hamstring graft, tensioned and secured correctly, is stronger and stiffer than all ten-millimeter patellar ligament grafts tested in previously reported studies[13,25]. Cooper et al.[13] reported the highest strength (2977 newtons) for a ten-millimeter patellar ligament graft.

We believe that clamping of a graft in the laboratory and fixation of a graft during a ligament reconstruction are similar processes. In the present study, the grafts were clamped and then subjected to tensile loads, whereas grafts *in vivo* are secured to bone and then subjected to physiological forces. The clinically relevant finding of our study is that it is necessary to apply equal tension to all strands of a hamstring graft during fixation to obtain optimum tensile properties of the graft. There must be equal tension on each strand of a combined four-strand graft during fixation if it is to have greater strength and stiffness than a two-strand semitendinosus graft. The present study demonstrated that all strands of a hamstring graft must be under equal tension for the composite to have its optimum biomechanical properties.

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