Determination of a Safe Range of Knee Flexion Angles for Fixation of the Grafts in Double-Bundle Anterior Cruciate Ligament Reconstruction

A Human Cadaveric Study

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Background: For anterior cruciate ligament reconstruction with a double-bundle procedure, one of the major concerns is to not predispose either one of the grafts to risk of failure by overloading.

Hypothesis: Knee flexion angles between 15° and 45° for anteromedial graft fixation and 15° for posterolateral graft fixation are safe for both grafts in double-bundle anterior cruciate ligament reconstruction.

Study Design: Controlled laboratory study.

Methods: Nine human cadaveric knees were tested. The double-bundle anterior cruciate ligament reconstruction was conducted with both grafts fixed at 15° of knee flexion (fixation protocol 15/15) and again with the anteromedial and posterolateral grafts fixed at 45° and 15° of knee flexion (fixation protocol 45/15). For both fixation protocols, the knee kinematics and the in situ forces of the reconstructed anterior cruciate ligament and its individual grafts were measured and collected under an anterior tibial load of 134 N and combined rotatory loads of 10 N·m of valgus and 5 N·m of internal tibial torque. The data from both fixation protocols were compared with those of an intact knee.

Results: In response to the 2 external loading conditions, both fixation protocols (15/15 and 45/15) could restore the knee kinematics to within 2 mm of the intact knee (although statistically significant differences were found between fixation protocol 15/15 and the intact knee) and the overall in situ forces in the grafts similar to the intact anterior cruciate ligament. In response to the 134-N anterior tibial load, the in situ forces in the anteromedial graft for both fixation protocols did not exceed those of the intact anteromedial bundle. But at 30° and 45° of knee flexion, the in situ forces for fixation protocol 15/15 were 20.7% and 22.1% lower, respectively, when compared with the intact anteromedial bundle. Under combined rotatory loads, the anteromedial graft for fixation protocol 15/15 had in situ forces that were 45% lower than the intact anteromedial bundle at 30° of knee flexion. The in situ force in the posterolateral graft for both fixation protocols did not exceed those of the intact posterolateral bundle, nor were they significantly different from the intact posterolateral bundle at any of the flexion angles tested.

Conclusion: Both fixation protocols restored knee kinematics without predisposing either graft to failure. Therefore, knee flexion angles between 15° and 45° for graft fixation were found to be safe for the anteromedial graft, while 15° of knee flexion was safe for the posterolateral graft.

Clinical Relevance: A range of knee flexion angles that is safe for the fixation of both grafts in double-bundle anterior cruciate ligament reconstruction was determined.

Keywords: double-bundle ACL reconstruction; graft fixation; flexion angle; knee kinematics; in situ force

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Double-bundle anterior cruciate ligament reconstruction (DB-ACLR) uses 2 separate grafts to replace the 2 functional bundles of the anterior cruciate ligament (ACL): the anteromedial (AM) bundle and the posterolateral (PL) bundle. This procedure was first introduced in the early 1980s and has been popularized in Japan and Europe during the last decade.[17,29] Laboratory studies from our research center have shown differences in the function of the 2 bundles of the ACL. When the knee is subjected to external loads such as an anterior tibial load (ATL), the AM and PL bundles share the load when the knee is near extension, while the AM bundle carries the majority of the load as the knee is flexed.[6,21] A follow-up study further showed that a single-bundle ACL reconstruction could sufficiently restore anterior tibial translation (ATT) in response to an ATL but became inadequate in response to a combined rotatory load (CRL) of valgus and internal/external rotation, especially when the knee was near extension.[25] On the other hand, a DB-ACLR was shown to better restore knee kinematics in comparison with a traditional single-bundle ACL reconstruction under CRL.[25]

One of the major issues in DB-ACLR centers on the variability of the knee flexion angles chosen for fixation of the AM and PL grafts. The range of angles used by surgeons varies widely, that is, from 10º to 90º for the AM graft and from full extension to 60º for the PL graft.[2,3,10,14,18,25,27] Some authors have advocated fixing both grafts at the same knee flexion angle, while others choose 2 separate knee flexion angles that correspond to the knee flexion angle at which each bundle carries the highest load.[7,10,18,28] Thus, a laboratory study was conducted recently that looked into whether and how the knee flexion angle for graft fixation affected the force distribution in the 2 grafts.[15] In the first fixation protocol, both the AM and PL grafts were fixed at 30° of knee flexion (fixation protocol 30/30). For the second fixation protocol, the AM graft was fixed at 60° of knee flexion, while the PL graft was fixed at full extension (fixation protocol 60/FE), which corresponds to the flexion angles at which each intact bundle carries higher loads.[6] For fixation protocol 30/30, the PL graft was overloaded by an average of 34% above the intact PL bundle under an applied 134-N ATL. When the reconstructed knee was subjected to CRLs of 10 N·m of valgus and 5 N·m of internal/external torque, even more alarming results were found, as the PL graft was overloaded by an average of 67%. For fixation protocol 60/FE, the AM graft was overloaded by an average of 46% compared with the intact AM bundle under a 134-N ATL.[15] These results clearly demonstrated that the knee flexion angles chosen for graft fixation could have a significant effect on the distribution of the in situ forces between the AM and PL grafts and that a poor choice could overload 1 of the grafts and predispose it to failure. The findings suggested that the AM graft should be fixed at a knee flexion angle of less than 60°, while the PL graft should be fixed closer to extension.

Based on the results of Miura et al,[15] a new research question was posed regarding what range of knee flexion angles would be safe for graft fixation in DB-ACLR. The objective of this study was to determine the knee flexion angles for graft fixation such that the in situ forces of the AM and PL grafts would be similar to those for their respective intact AM and PL bundles without exceeding the intact values. The 2 angles chosen for AM graft fixation were 15° and 45° of knee flexion, while the PL graft was fixed at 15° of knee flexion. The previous study concluded that the PL graft should be fixed between full extension and 30° of knee flexion. Therefore, the knee flexion angle for fixation of the PL graft was kept consistent at 15° of knee flexion. For fixation of the AM graft, it was suggested that the knee flexion angle should be less than 60°; however, an actual range of knee flexion angles for AM graft fixation was undetermined. Thus, 45° of knee flexion was chosen for the upper bound, and 15° of knee flexion was chosen as the lower bound, which then provided us with a range from 15° to 45° for the AM graft.

We hypothesized that fixation of the AM graft at either 15° or 45°, and the PL graft near extension, ie, 15°, would keep the in situ forces in the AM and PL grafts from exceeding those of their respective intact bundles when the knee is subjected to ATL and CRL. The knee kinematics and the in situ forces in the AM and PL grafts after the 2 fixation protocols were measured using a robotic/UFS testing system.[5] The loading conditions were applied between full extension and 120° of knee flexion, and the resulting 5 degrees of freedom (DOF) kinematics and in situ forces of the 2 bundles of the intact ACL, as well as those of the 2 grafts, were measured and compared.

MATERIALS AND METHODS

Nine fresh-frozen human cadaveric knees (age, 43.3 ± 8.1 y) were tested for this study based on a power analysis. Roentgenograms of specimens were taken and examined to ensure there was no evidence of osteoarthritis or bony abnormalities. Specimens were stored in airtight plastic bags at –20°C until 24 hours before testing, when they were thawed at room temperature.[16,24] Arthroscopic examination of the knee joint was performed to confirm the presence of an intact and functional ACL.

The semitendinosus and gracilis tendons were chosen as ACL replacement grafts, which were harvested using a tendon stripper from each knee through an approximately 4-cm-long longitudinal incision on the anteromedial portion of the proximal tibia, 2 cm distal to the tibial tuberosity. The grafts were then wrapped in saline-soaked gauze to prevent dehydration.

In preparation for testing, all soft tissue was removed approximately 10 cm away from the joint line on both the femur and the tibia, while leaving the knee joint intact. The fibula was fixed to the tibia with a metal screw to maintain its anatomic position. The tibia and the femur were each secured within custom-made aluminum cylinders by using an epoxy compound (Fibre Glass-Evercoat, Cincinnati, Ohio) with transfixing bolts. The specimen was then mounted in a robotic universal force moment sensor (UFS) testing system.[4,5,13,20] The femoral side was rigidly mounted to the base of the robotic manipulator (PUMA Model 726, Unimate Inc, Danbury, Conn), while the tibial side was attached to the end-effector of the robotic manipulator via a load cell (Model 4015, JR3 Inc, Woodland, Calif) (Figure 1). The robotic manipulator has a position and orientation repeatability of less than 0.2 mm and 0.2°, respectively.[20] The UFS is capable of measuring 3 forces and 3 moments in a Cartesian coordinate system fixed with respect to the sensor. Using the robotic/UFS testing system, the knee kinematics and the in situ forces in the

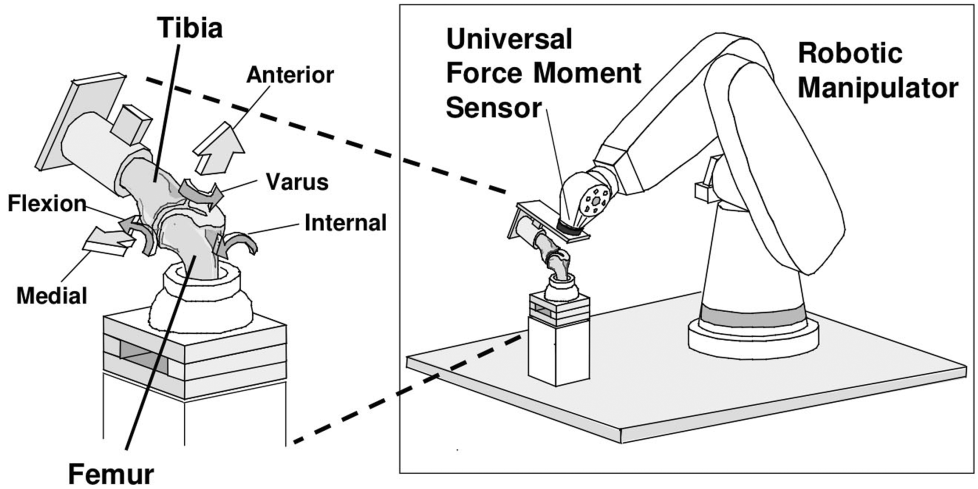

Figure 1. The robotic universal force moment sensor (UFS) testing system with cadaveric knee specimen.

intact ACL and the ACL graft, as well as the in situ forces in the AM and PL bundles and their respective replacement grafts, were obtained.

Throughout the experiment, the knees were kept moist with 0.9% saline solution. The sequence of tests performed and the data acquired are outlined in Figure 2. The path of passive flexion-extension of the intact knee from full extension to 120° of flexion was first determined by the robotic/UFS testing system in 1° increments by means of minimizing all the external forces and moments. This path serves as the reference position from which external loads are applied and kinematics data are collected. Two external loading conditions were applied to the knee: (1) a 134-N ATL with the knee at full extension, 15°, 30°, 45°, 90°, and 120° of flexion; and (2) a combined 10 N·m of valgus torque and 5 N·m of internal-external tibial torque at 15° and 30° of knee flexion. The ATL simulates clinical examinations such as the anterior drawer and the Lachman tests, which are commonly used for the diagnosis of ACL deficiency.[19,22,23] The CRL statically simulates the pivot-shift test, which is another examination used to determine knee instability, especially rotatory instability due to ACL injury. The 5 DOF kinematics of the intact knee (ie, anterior-posterior, medial-lateral, and proximal-distal translations, as well as internal-external and varus-valgus rotations) were recorded. The AM and PL bundles of the ACL were then separated and transected in turn through a medial parapatellar arthrotomy using a previously described technique,[8] which resulted in 2 knee states, namely, the bundle-deficient knee and the ACL-deficient knee. The cutting of the bundles was randomized among knees. The kinematics of the intact knee was replayed after each new knee state. The corresponding resultant forces were measured. Based on the principle of superposition, the magnitude of the vector difference of the 2 resulting force vectors under the intact and the ACL-deficient knee states represents the in situ force in the ACL. Similarly, the force distribution between the AM and PL bundles could be determined by comparing the force vectors obtained under the bundle-deficient and ACL-deficient knee states. To evaluate changes in knee kinematics associated with ACL deficiency, the same 2 loading conditions were applied to the ACL-deficient knee, and the resulting 5 DOF knee kinematics were measured.

Double-bundle ACLR was performed using 2 femoral tunnels and a single tibial tunnel by a medial arthrotomy to better visualize the insertions of the individual bundles of the ACL.[25,26] The medial arthrotomy was done during specimen preparation; therefore, any effect of performing this procedure was consistent throughout the experiment. Femoral tunnels were drilled on the footprints of the 2 bundles, which corresponded roughly to around the 11-o'clock position for the AM graft and around the 9-o'clock position for the PL graft for a right knee and the 1-o'clock and 3-o'clock positions, respectively, for a left knee. The diameter of the femoral tunnels for the AM and PL grafts was chosen according to the graft size (range, 6-8 mm for AM, and range, 5-7 mm for PL). Both the AM and PL femoral tunnels were positioned using a Kirschner wire at the center of the insertion of each bundle of the ACL by visual inspection of the remnants of the transected bundles. The femoral tunnels were first drilled 30 mm deep inside the joint using a cannulated drill bit chosen according to the graft diameter; then the tunnels were drilled through with a 4.5-mm-diameter cannulated drill (EndoButton Drill, Acufex, Smith & Nephew, Andover, Mass). A single tibial tunnel was placed using a Protac tibial guide set at 55°. The diameter of the tibial tunnel was equal to the diameter of the AM and PL combined graft (range, 7.5-10 mm). Looped semitendinosus tendon and gracilis tendon were used for the AM and the PL grafts, respectively. For each graft, the femoral side was fixed first using an EndoButton CL[18] (Smith & Nephew). The grafts were then pulled through the tibial tunnel, and the knee was preconditioned by moving the knee through 5 cycles of the full range of knee flexion while applying a 22-N pretension to each graft. Finally, the tibial side was fixed using 2 spiked washers and 2 screws. For fixation protocol 15/15,

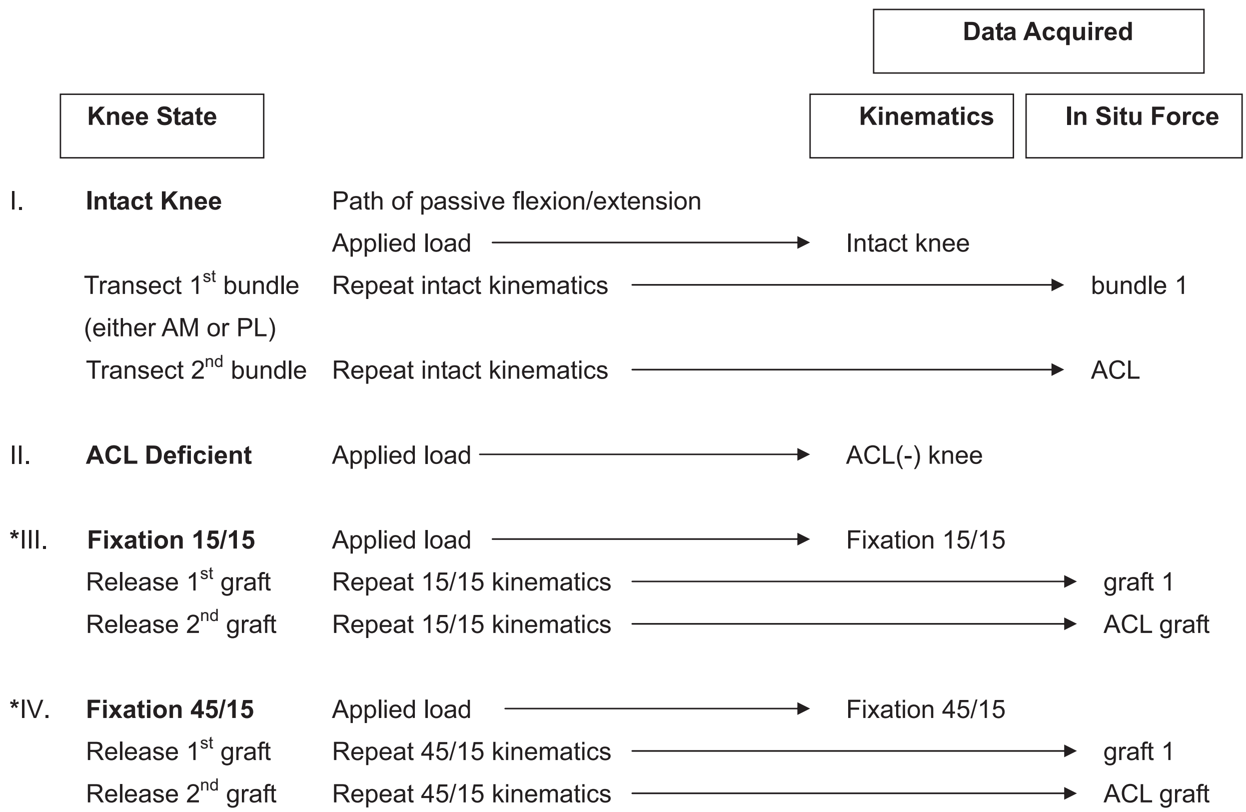

Figure 2. An experimental protocol and the corresponding data acquired. *The order of III and IV were randomized.

both grafts were fixed at the same knee flexion angle of 15°, while for fixation protocol 45/15, the AM graft was fixed at 45° of knee flexion and the PL graft at 15°. Each graft was fixed while a 67-N posterior tibial load and 22 N of initial graft tension were maintained. Previous studies have shown that applying 67 N of posterior load during the fixation of the grafts can more closely restore the knee kinematics and the in situ force in the ACL graft to those of the intact knee.[9] The initial graft tension was chosen according to a previous publication that determined 44 N was enough to restore the knee kinematics without overconstraining the knee joint.[1] Therefore, 22 N of initial graft tension was applied to each graft, which totaled 44 N of initial graft tension. The order of graft fixation was randomized among knees.

After each reconstruction, the same 2 external loading conditions applied to the intact knee were repeated on the ACL-reconstructed knee. The corresponding 5 DOF knee kinematics were compared with those of the intact knee and the ACL-deficient knee. The force distributions in the AM and PL grafts were obtained by releasing the AM graft and the PL graft in turn and replaying the knee kinematics of the reconstructed knee.

On the basis of our previous data, a power analysis was performed (power = .80, significance level = .05), so differences of 3 mm for ATT and 20 N for in situ force measurements could be detected. It was determined that 9 knees were required for this study. Because all variables were measured on the same specimen, statistical analysis of the ATT and in situ forces was performed using a 1-factor repeated measures analysis of variance (ANOVA), with knee state as the factor. This analysis has the advantage of being sensitive to relatively small changes occurring within an individual knee and minimizing the effects of interspecimen variability. A Bonferroni adjustment was done to evaluate the effects of ACL reconstruction at specific angles of knee flexion. Statistical significance was set at $P < .05$.

RESULTS

Anterior Tibial Load

Under a 134-N ATL, the ATT of the intact knee ranged from 4.0 ± 0.9 mm at full extension to 7.6 ± 2.1 mm at 30° of knee flexion (Table 1). After the ACL was transected, the ATT more than doubled for all the flexion angles tested, measuring as high as 17.2 ± 3.0 mm at 30° ($P < .05$). After ACL reconstruction, the ATT was restored to within 2.2 mm of the intact knee for both fixation protocols 15/15 and 45/15 (Table 1). For example, the ATT at 30° of knee flexion was 9.8 ± 2.1 mm for fixation protocol 15/15 and 9.1 ± 2.4 mm for fixation protocol 45/15. Although the maximum

TABLE 1
Anterior Tibial Translation (mm) in Response to a 134-N Anterior Tibial Load (Mean ± SD)

Flexion Angle (degrees)	Intact Knee (mm)	ACL-Deficient Knee (mm)	ACL-Reconstructed Knee (mm) Fixation 45/15	ACL-Reconstructed Knee (mm) Fixation 15/15
Full extension	4.0 ± 0.9	[a]8.6 ± 2.1	4.4 ± 1.4	4.8 ± 1.4
15	6.5 ± 1.6	[a]15.3 ± 2.4	7.7 ± 2.1	[a]8.3 ± 2.0
30	7.6 ± 2.1	[a]17.2 ± 3.0	9.1 ± 2.4	[a]9.8 ± 2.1
45	7.7 ± 2.2	[a]16.3 ± 3.7	9.2 ± 2.1	[a]9.9 ± 1.9
90	5.9 ± 2.1	[a]11.1 ± 3.5	6.7 ± 1.8	[a]7.3 ± 1.8
120	6.3 ± 1.9	[a]11.6 ± 2.7	6.8 ± 1.7	7.4 ± 1.9

[a]$P < .05$ compared with the intact knee.

TABLE 2
In Situ Force (N) of the Anterior Cruciate Ligament (ACL) and ACL Grafts in Response to a 134-N Anterior Tibial Load (Mean ± SD)

Flexion Angle (degrees)	Intact ACL (N)	ACL Grafts Fixation 45/15 (N)	ACL Grafts Fixation 15/15 (N)
Full extension	90 ± 23	82 ± 28	73 ± 24
15	119 ± 20	111 ± 15	107 ± 16
30	116 ± 14	109 ± 17	103 ± 22
45	103 ± 15	93 ± 18	86 ± 21
90	79 ± 14	70 ± 24	62 ± 19
120	76 ± 14	66 ± 19	63 ± 18

anterior laxity was only 2.2 mm, statistically significant differences were found between fixation protocol 15/15 and the intact knee.

Shown in Table 2 are the overall in situ forces of the intact ACL and the AM and PL bundles as well as the AM and PL grafts for both fixation protocols 15/15 and 45/15. In response to the 134-N ATL, the in situ force in the intact ACL ranged from 76 ± 14 N at 120° to 119 ± 20 N at 15°. For fixation protocol 15/15, the corresponding values for the combined grafts were 63 ± 18 N to 107 ± 16 N and were not statistically different compared with the intact knee ($P > .05$). Similarly, for fixation protocol 45/15, the in situ force for the combined ACL grafts was also not different from those of the intact ACL ($P > .05$). Furthermore, no statistical differences were found between the 2 fixation protocols, ie, 15/15 versus 45/15 ($P > .05$).

Results for the in situ force in the AM and PL bundles and their respective individual grafts were also obtained and compared. The intact AM bundle ranged from 46 ± 25 N at full extension to 79 ± 21 N at 45° in response to the 134-N ATL (Figure 3). The corresponding values for the AM graft in each fixation protocol were 31 ± 16 N at full extension to 61 ± 20 N at 45° for fixation protocol 15/15,

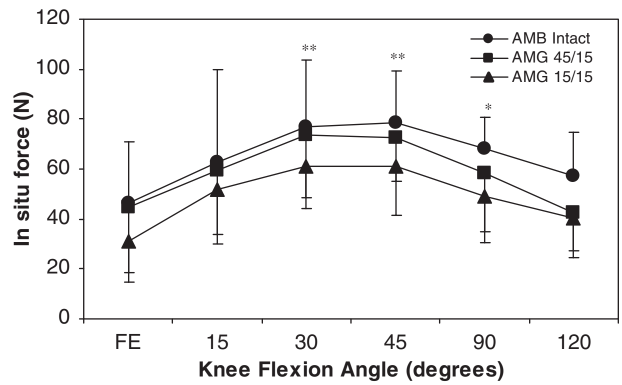

Figure 3. In situ force in the intact anteromedial bundle (AMB) and AM graft (AMG) in response to 134-N anterior tibial load for the 2 different fixation protocols. *$P < .05$ compared with AMB; **$P < .05$ between fixation protocol 45/15 and fixation protocol 15/15.

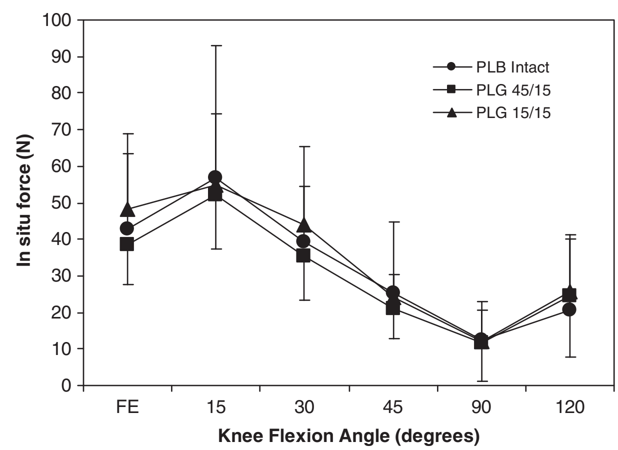

Figure 4. In situ force of the intact posterolateral bundle (PLB) and PL graft (PLG) in response to 134-N anterior tibial load for the 2 different fixation protocols.

and from 45 ± 26 N to 73 ± 17 N for fixation protocol 45/15, respectively ($P > .05$). Compared with the intact knee, the in situ forces for fixation protocol 15/15 were 79.3% and 77.9% of the intact AM bundle values at 30° and 45° of knee flexion, respectively. When comparing the 2 fixation protocols with each other, the in situ forces in the AM graft for fixation protocol 15/15 were significantly lower than those for fixation protocol 45/15, at 30° and 45° of knee flexion.

The in situ forces in the intact PL bundle ranged from 57 ± 37 N at 15° to 13 ± 11 N at 90° of knee flexion. Those for the PL graft ranged from 55 ± 20 N at 15° to 12 ± 9 N at 90° of knee flexion for fixation protocol 15/15, and from 52 ± 15 N to 12 ± 11 N for fixation protocol 45/15 (Figure 4). There was no statistically significant difference between the PL grafts for both fixation protocols with those for the intact PL bundle ($P > .05$). Also, there was no difference in between the grafts of the 2 fixation protocols from full extension to full flexion ($P > .05$).

TABLE 3
Coupled Anterior Tibial Translation (mm) in Response to Combined Rotatory Loads (Mean ± SD)

Flexion Angle (degrees)	Intact Knee (mm)	ACL-Deficient Knee (mm)	ACL-Reconstructed Knee (mm)	
			Fixation 45/15	Fixation 15/15
15	4.5 ± 3.9	[a]10.1 ± 5.1	5.7 ± 4.4	[a,b]6.1 ± 4.4
30	6.3 ± 4.4	[a]10.9 ± 5.1	7.4 ± 4.6	[a,b]7.7 ± 4.7

[a]$P < .05$ compared with the intact knee.
[b]$P < .05$ compared with the ACL-deficient knee.

Combined Rotatory Loads

Under the CRLs, the coupled ATT of the intact knee was 4.5 ± 3.7 mm at 15° and 6.3 ± 4.1 mm at 30° of knee flexion, while the internal tibial rotation (ITR) was 15.1° ± 3.4° and 17.2° ± 3.6°, respectively (Table 3). After transection of the ACL, the ATT values increased significantly to 10.1 ± 4.8 mm and 10.9 ± 4.8 mm at 15° and 30°, respectively ($P < .05$). After ACL reconstruction with fixation protocol 15/15, the ATT was 6.1 ± 4.2 mm at 15° and 7.7 ± 4.4 mm at 30°, while the ITR was 16.8° ± 3.1° and 18.5° ± 3.3°, respectively, and was statistically higher than that of the intact knee. Results obtained for fixation protocol 45/15 were comparable with the intact knee as there were no significant changes in ATT ($P > .05$) (Table 3).

Under CRL, the in situ force of the intact ACL was 82 ± 17 N at 15° and 70 ± 30 N at 30° of knee flexion. The corresponding values for the combined grafts for fixation protocol 15/15 were 65 ± 24 N and 54 ± 30 N, while for fixation protocol 45/15, the values were 72 ± 19 N and 54 ± 22 N, respectively. As in the case of the applied ATL, no statistically significant differences were found between the in situ force of the combined ACL grafts for both the fixation protocols and the intact ACL.

Results for the in situ force in the intact AM and PL bundles and their respective individual grafts were also obtained and compared. The in situ force in the intact AM bundle was 41 ± 15 N at 15° and 41 ± 19 N at 30° of knee flexion. The corresponding values for the AM graft for fixation protocol 15/15 were 26 ± 11 N at 15° and 22 ± 13 N at 30° of knee flexion, while for fixation protocol 45/15, they were 39 ± 18 N at 15° and 29 ± 14 N at 30° of knee flexion (Figure 5). As in the case of the applied ATL, the in situ force in the AM graft for fixation protocol 15/15 was 45% lower than that of the intact AM bundle at 30° of knee flexion ($P < .05$). No statistical difference was found between the AM graft for fixation protocol 45/15 and the intact AM bundle at either of the flexion angles tested. The in situ forces in the intact PL bundle were 42 ± 22 N at 15° and 30 ± 18 N at 30° of knee flexion. The in situ force in the PL graft did not exceed those of the intact PL bundle for both fixation protocols. The corresponding values for the PL graft for fixation protocol 15/15 were 40 ± 17 N at 15° and 30 ± 15 N at 30° of knee flexion, while for fixation protocol 45/15,

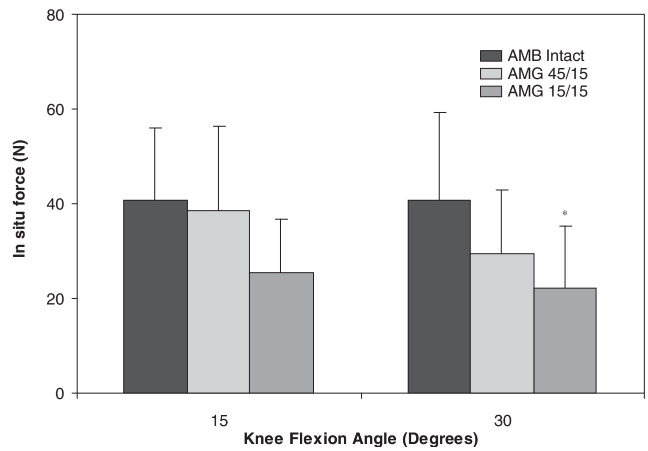

Figure 5. In situ force of the intact anteromedial bundle (AMB) and AM graft (AMG) in response to combined rotatory loads of 10 N·m of valgus and 5 N·m of internal torques for the 2 different fixation protocols. *$P < .05$ compared with AMB.

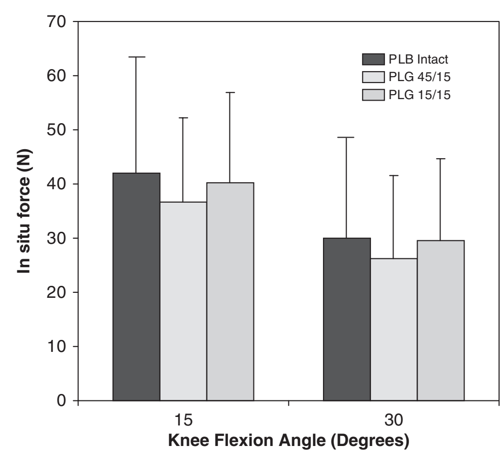

Figure 6. In situ force of the intact posterolateral bundle (PLB) and PL graft (PLG) in response to combined rotatory loads of 10 N·m of valgus and 5 N·m of internal torques for the 2 different fixation protocols.

they were 37 ± 16 N and 26 ± 15 N, respectively (Figure 6). There was no statistical difference between the 2 PL grafts and the intact PL bundle as well as in between the grafts of the 2 fixation protocols for both flexion angles tested under the CRLs ($P > .05$).

DISCUSSION

In this human cadaveric study, quantitative data on knee kinematics in response to an ATL and a CRL, as well as the corresponding in situ forces in the AM and PL grafts, after a DB-ACLR for 2 fixation protocols were determined by means of a robotic/UFS testing system. The advantages of this testing system include the measurement of kinematics while the knee undergoes unrestricted motion in multiple degrees of freedom. More importantly, this methodology allowed the experimental data for an intact knee, ACL-deficient knee, and ACL-reconstructed knee with fixation protocols 15/15 and 45/15 to be obtained from the same cadaveric specimen. This procedure eliminated inter-specimen variation and, as a result, significantly increased the statistical power of the data by using repeated measures ANOVA.[4]

The objective of this study was achieved as it was found that both fixation protocols (15/15 and 45/15) under the 2 external loading conditions could restore the knee kinematics to within 2.2 mm of those of the intact knee as well as yield overall in situ forces comparable to the intact ACL. Although statistically significant kinematic differences were found between fixation protocol 15/15 and the intact knee, the maximum difference of 2.2 mm is still within the clinically acceptable range. The knee kinematics and in situ forces of the ACL obtained in our study were similar to those published in the literature.[11,12,15,27] Neither of the grafts had their in situ forces exceed those of their respective intact AM and PL bundles of the ACL. However, under ATL, the AM graft in situ forces for fixation protocol 15/15 were significantly different (79.3% and 77.9% of the intact AM bundle) at 30° and 45° of knee flexion. This data suggest that fixation protocol 45/15 could be more favorable because in addition to restoring knee kinematics, the in situ forces were closer to the intact knee at all knee flexion angles tested under both loading conditions. This could be due to the fact that the intact AM bundle carries higher loads at around 45° of knee flexion, while the intact PL bundle carries higher loads at around 15° of knee flexion.

A previous study from our research center found that for graft fixation in DB-ACLR, the fixation of the AM and PL bundles at 60/FE and 30/30, respectively, resulted in the in situ forces of the AM graft and the PL graft to be above those of the AM and PL bundles, respectively. With that knowledge, a strategy was chosen to examine a range of knee flexion angles for safe graft fixation during DB-ACLR by varying the flexion angle for the AM graft while keeping the flexion angle for the PL graft at 15°. This is necessary because the 2 grafts are interactive during knee motion, and thus there exists an infinite combination of knee flexion angles for the fixation of each graft. Therefore, only a range of knee flexion angles was sought. In addition, the practicality of the knee flexion angles used during surgery was kept in mind. Our data suggest that when the PL graft is fixed at 15° of knee flexion, the AM graft can be fixed between 15° and 45°, supporting our hypothesis because the in situ forces in the AM and PL grafts were indeed lower than those of their respective bundles of the intact ACL.

It should be noted that the external loads applied to the knee are only representative of those during clinical examinations, and the magnitudes are significantly lower than what would be seen during activities of daily living. In addition, because this is an in vitro laboratory study, many in vivo variables, such as tunnel-graft healing, tunnel motion, graft remodeling, and graft relaxation, were not taken into account as this type of study can only observe the time-zero conditions. Therefore, we recommend further in vitro and in vivo studies in order to have a better idea of the behavior of the 2-bundle complex of the ACL.

This study looked into the force distribution of the AM and PL graft after DB-ACLR under simulated clinical examination maneuvers and found that for the fixation protocols tested, both the AM and PL grafts did not carry more load than their respective intact bundles, which puts the grafts at a lower risk of graft failure during an in vivo loading condition in which the magnitude of the force would be much higher. Thus, these results could serve as guidelines for DB-ACLR.

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REFERENCES

1. Abramowitch SD, Papageorgiou CD, Withrow JD, Gilbert TW, Woo SL. The effect of initial graft tension on the biomechanical properties of a healing ACL replacement graft: a study in goats. *J Orthop Res.* 2003;21:708-715.
2. Adachi N, Ochi M, Uchio Y, Iwasa J, Kuriwaka M, Ito Y. Reconstruction of the anterior cruciate ligament. Single- versus double-bundle multi-stranded hamstring tendons. *J Bone Joint Surg Br.* 2004;86:515-520.
3. Aglietti P, Giron F, Buzzi R, Biddau F, Sasso F. Anterior cruciate ligament reconstruction: bone-patellar tendon-bone compared with double semitendinosus and gracilis tendon grafts. A prospective, randomized clinical trial. *J Bone Joint Surg Am.* 2004;86:2143-2155.
4. Fujie H, Livesay GA, Woo SL-Y, Kashiwaguchi S, Blomstrom G. The use of a universal force-moment sensor to determine in situ forces in ligaments: a new methodology. *J Biomech Eng.* 1995;117:1-7.
5. Fujie H, Mabuchi K, Woo SL-Y, Livesay GA, Arai S, Tsukamoto Y. The use of robotics technology to study human joint kinematics: a new methodology. *J Biomech Eng.* 1993;115:211-217.
6. Gabriel MT, Wong EK, Woo SL-Y, Yagi M, Debski RE. Distribution of in situ forces in the anterior cruciate ligament in response to rotatory loads. *J Orthop Res.* 2004;22:85-89.
7. Hamada M, Shino K, Horibe S, et al. Single- versus bi-socket anterior cruciate ligament reconstruction using autogenous multiple-stranded hamstring tendons with EndoButton femoral fixation: a prospective study. *Arthroscopy.* 2001;17:801-807.
8. Harner CD, Giffin JR, Dunteman RC, Annunziata CC, Friedman MJ. Evaluation and treatment of recurrent instability after anterior cruciate ligament reconstruction. *Instr Course Lect.* 2001;50:463-474.
9. Hoher J, Scheffler S, Weiler A. Graft choice and graft fixation in PCL reconstruction. *Knee Surg Sports Traumatol Arthrosc.* 2003;11:297-306.
10. Ishibashi Y, Tsuda E, Tazawa K, Sato H, Toh S. Intraoperative evaluation of the anatomical double-bundle anterior cruciate ligament reconstruction with the OrthoPilot navigation system. *Orthopedics.* 2005;28(10 Suppl):S1277-1282.
11. Kanamori A, Woo SL-Y, Ma CB, et al. The forces in the anterior cruciate ligament and knee kinematics during a simulated pivot-shift test: a human cadaveric study using robotic technology. *Arthroscopy.* 2000;16:633-639.
12. Kanamori A, Zeminski J, Rudy TW, Li G, Fu FH, Woo SL-Y. The effect of axial tibial torque on the function of the anterior cruciate ligament: a biomechanical study of a simulated pivot-shift test. *Arthroscopy.* 2002;18:394-398.

13. Livesay GA, Fujie H, Kashiwaguchi S, Morrow DA, Fu FH, Woo SL. Determination of the in situ forces and force distribution within the human anterior cruciate ligament. *Ann Biomed Eng.* 1995;23:467-474.
14. Marcacci M, Molgora AP, Zaffagnini S, Vascellari A, Iacono F, Presti ML. Anatomic double-bundle anterior cruciate ligament reconstruction with hamstrings. *Arthroscopy.* 2003;19:540-546.
15. Miura K, Woo SL-Y, Brinkley R, Fu YC, Noorani S. Effects of knee flexion angles for graft fixation on force distribution in double-bundle anterior cruciate ligament grafts. *Am J Sports Med.* 2006;34:577-585.
16. Moon DK, Woo SL-Y, Takakura Y, Gabriel MT, Abramowitch SD. The effects of refreezing on the viscoelastic and tensile properties of ligaments. *J Biomech.* 2006;39:1153-1157.
17. Mott HW. Semitendinosus anatomic reconstruction for cruciate ligament insufficiency. *Clin Orthop Relat Res.* 1983;172:90-92.
18. Muneta T, Sekiya I, Yagishita K, Ogiuchi T, Yamamoto H, Shinomiya K. Two-bundle reconstruction of the anterior cruciate ligament using semitendinosus tendon with EndoButtons: operative technique and preliminary results. *Arthroscopy.* 1999;15:618-624.
19. Nielsen S, Helmig P. Instability of knees with ligament lesions. Cadaver studies of the anterior cruciate ligament. *Acta Orthop Scand.* 1985;56:426-429.
20. Rudy TW, Livesay GA, Woo SL-Y, Fu FH. A combined robotic/universal force sensor approach to determine in situ forces of knee ligaments. *J Biomech.* 1996;29:1357-1360.
21. Sakane M, Fox RJ, Woo SL-Y, Livesay GA, Li G, Fu FH. In situ forces in the anterior cruciate ligament and its bundles in response to anterior tibial loads. *J Orthop Res.* 1997;15:285-293.
22. Sullivan D, Levy IM, Sheskier S, Torzilli PA, Warren RF. Medial restraints to anterior-posterior motion of the knee. *J Bone Joint Surg Am.* 1984;66:930-936.
23. Torzilli PA, Greenberg RL, Insall J. An in vivo biomechanical evaluation of anterior-posterior motion of the knee. Roentgenographic measurement technique, stress machine, and stable population. *J Bone Joint Surg Am.* 1981;63:960-968.
24. Woo SL-Y, Orlando CA, Camp JF, Akeson WH. Effects of postmortem storage by freezing on ligament tensile behavior. *J Biomech.* 1986; 19:399-404.
25. Yagi M, Wong EK, Kanamori A, Fu FH, Woo SL-Y. Biomechanical analysis of an anatomic anterior cruciate ligament reconstruction. *Am J Sports Med.* 2002;30:660-666.
26. Yamamoto Y, Hsu WH, Woo SL-Y, Van Scyoc AH, Takakura Y, Debski RE. Knee stability and graft function after anterior cruciate ligament reconstruction: a comparison of a lateral and an anatomical femoral tunnel placement. *Am J Sports Med.* 2004;32:1825-1832.
27. Yasuda K, Kondo E, Ichiyama H, et al. Anatomic reconstruction of the anteromedial and posterolateral bundles of the anterior cruciate ligament using hamstring tendon grafts. *Arthroscopy.* 2004;20:1015-1025.
28. Yasuda K, Kondo E, Ichiyama H, Tanabe Y, Tohyama H. Clinical evaluation of anatomic double-bundle anterior cruciate ligament reconstruction procedure using hamstring tendon grafts: comparisons among 3 different procedures. *Arthroscopy.* 2006;22:240-251.
29. Zaricznyj B. Reconstruction of the anterior cruciate ligament of the knee using a doubled tendon graft. *Clin Orthop Relat Res.* 1987; 220:162-175.