# The effect of medial collateral ligament insufficiency on the reconstructed anterior cruciate ligament

## A study in the rabbit

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**ABSTRACT** **The treatment for severe combined anterior cruciate ligament (ACL) and medial collateral ligament (MCL) ruptures is disputed. Using a rabbit model, we examined the effect of insufficiency of medial structures on the reconstructed ACL in combined ACL and MCL injury. 40 rabbits were divided into 2 groups. In both groups, ACL was subjected to in situ freeze-thaw treatment. In group F, only freeze-thaw treatment of ACL was given. In group FM, partial resection of MCL was also done. We killed 5 rabbits on each of 4 occasions: immediately after the operation (time 0), at 6, 12 and 24 weeks postoperatively. At each time, we measured valgus instability and mechanical properties of the ACL.**

**Valgus instability in group FM persisted from time 0 to 24 weeks, and was significantly greater than that in group F. The tensile strength and tangent modulus of the ACL in group FM were lower than those in group F. We found that continuous valgus instability reduces the mechanical properties of the in situ frozen ACL.**

Experimental and clinical studies have shown that an isolated injury of the medial collateral ligament (MCL) can be treated nonsurgically (Fetto and Marshall 1978, Indelicato 1983, Reider et al. 1993, Lundberg and Messner 1996). In complex ligament injuries of the knee joint, however, the treatment for severe combined ruptures of the anterior cruciate ligament (ACL) and MCL is disputed. The treatments for such injuries include surgical restoration of all ligamentous structures (Warren and Marshall 1978, Anderson and Gillquist 1992), surgical reconstruction of the ACL alone (Ballmer et al. 1991, Shelbourne and Porter 1992, Sembell et al. 1996), surgical repair of the medial complex (Hughston 1994), or nonsurgical treatment of both the ACL and medial structures (Jokl et al. 1984, Mok and Good 1989). The results of such treatments vary with the degree of damage, the period from injury to operation and postoperative rehabilitation. When ACL reconstruction alone is performed in cases with less valgus instability, a good result may be expected. But in grade III injury, especially chronic cases, the ACL graft will deteriorate, because the ACL is the second hindrance to valgus and rotatory stress (Inoue et al. 1987). No experimental data are available on the effect of valgus instability on remodeling of the reconstructed ACL. We studied how insufficiency of the MCL affects the reconstructed ACL in a rabbit model.

## Animals and methods

We used 40 mature male Japanese albino rabbits weighing 3.4 ± 0.2 kg. Throughout the experiments, all rabbits were allowed free access to standard feed and water. They were housed with a 12–12 light-dark cycle at 22–24 °C. We used an in situ freeze-thaw model (Jackson et al. 1991, Katuragi et al. 2000) to simulate the remodeling of an autogenous graft of the ACL, without the influence of a surgical technique. In this model, the cells in the ACL were killed, and the vascular supply was interrupted.

### *Surgical technique*

The operations were performed under intravenous pentobarbital anesthesia (20 mg/kg body weight), using sterile technique. The right knee joint was approached via a lateral parapatellar incision. The patella was displaced medially and the infrapatellar fat pad was mobilized and retracted to expose the entire ACL. All soft tissues were removed from the ACL to its femoral insertion. A wooden blade was inserted between the posterior cruciate ligament (PCL) and the ACL, to protect the PCL from freezing. The ACL was frozen in situ by liquid nitrogen (Jackson et al. 1991, Katsuragi et al. 2000). Using a specially-prepared freeze-probe, liquid nitrogen was applied to the ACL for 2 minutes. This was followed by saline solution for 2 minutes to thaw the ACL. The freeze-thaw cycles were repeated 3 times.

The rabbits were divided into 2 groups. In one, the right ACL was subjected to 3 freeze-thaw cycles (group F, n = 20), while in the other, the ACL was treated in the same way, but part of the MCL was resected to induce continuous valgus instability (group FM, n = 20). In group FM, the MCL was exposed and separated from the underlying medial meniscus by sharp dissection. The proximal half of the MCL was resected with a #11 scalpel blade, but its origin was not removed. The posteromedial structures were released from the medial meniscus and the tibia. In all animals, the contralateral side was not operated on, but served as the control.

The joint capsule and skin were sutured. In both groups, we killed 5 rabbits with an overdose of intravenous sodium pentobarbital on of 4 occasions: time 0 (immediately), 6, 12 and 24 weeks after the operation. In both groups, at time 0, we measured valgus instability alone, and at 6, 12, and 24 weeks, valgus instability, cross-sectional area and tensile strength of the ACL.

### *Mechanical testing*

The hind limbs were removed at about 50 mm above and below the knee. Both limbs were tested immediately after the animals were killed. The muscle and fascia surrounding the joint capsule were removed, leaving the menisci, collateral and cruciate ligaments intact.

### *Measurement of valgus laxity*

The femur was secured with 2 bent metal plates and 2 1.5-mm Kirschner wires to an acrylic plate with the knee at 30° of flexion. A valgus moment of 25 Nm was applied to the distal tibia. A radiograph was taken when the stress was applied. The angle between the distal joint surface of the femur and proximal to the tibia was measured by image analyzing software.

### *Cross-sectional area measurement*

All structures, apart from the ACL, were removed, yielding a femur-ACL-tibia complex (FATC). The medial femoral condyle and anterior portion of the distal femur were resected with a reciprocating bone saw to measure the cross-sectional area and for visualization during tensile testing. Each specimen was mounted on an originally-designed device and suspended in a saline bath at 37 °C, with a 50 g weight at the tibial end. The ligament was rotated at 5° angular increments through 180 °, and tracked by a digital video camera. In each image, we measured the the width of the center of the ACL, using image analyzing software, and the cross-sectional area.

### *Tensile testing*

The fibers in the lateral half of the ACL and the posterior portion of the medial fibers were transected and removed to permit a more uniform distribution of stress in the ligament during tensile testing (Woo et al. 1992). The cross-sectional area of the ACL was measured in the same manner. The femur and tibia were cemented into aluminum pots with polymethylmethacrylate; they were then fixed in originally-designed clamps, mounted on an Autograf Model AG-B, and adjusted with the longitudinal axis of the ACL, which was aligned with the load axis of the machine, the knee in 45° flexion, and the tibia was internally rotated to 90°. We measured strain by placing 2 dye lines on the midsubstance of the ACL, about 1 cm apart. Each specimen was preconditioned by applying a load of 1 N for 2 minutes and then loading to failure at a rate of 10 mm/min to obtain a load-time curve. During the test, saline was dropped on the specimen to prevent it from drying. All tests were tracked by digital video camera to determine the strain. The failure modes of the FATCs were noted.

**Table 1. Side-to-side difference (degrees) in valgus laxity, mean (SD)**

| | Postoperative weeks | | | |
|---|---|---|---|---|
| Group | 0 | 6 | 12 | 24 |
| F | –0.56 (1.3) | 0.01 (0.4) | 0.04 (0.3) | –0.2 (1.0) |
| FM | 8.3 (2.6) | 8.8 (2.4) | 8.0 (4.1) | 8.9 (4.2) |

### *Tensile-strain determination*

We measured the strain in the ligament substance with the image analyzing system, using a digital video camera. Static images were taken every second until failure. In each image, the distance between the 2 dye lines on the ligament (l) was measured, and a strain-time curve determined. The initial distance ($l_0$) of the ligament was determined at 0.5 seconds before the test. The strain of the ligament was defined as $((l-l_0)/l_0)$. From the load-time and strain-time curve, we determined the stress-strain curve.

This curve was used to measure the slope of the linear portion and tangent modulus. We also calculated the ratio of the experimental to the control value for each specimen.

### *Statistics*

All data, expressed as means (SD) inthe groups, were combined with a two-way factorial ANOVA. The Mann-Whitney U-test was used to compare the differences in mechanical testing between the groups for each week, and those between weeks in each group. The findings were considered significant at $p < 0.05$.

## Results

### *Valgus instability*

Immediately after the operation, the side-to-side difference in valgus instability in group FM was 8.3° (2.6°) (Table 1). The instability persisted for 24 weeks. It was greater in group FM than that in group F ($p < 0.0001$).

### *Cross sectional area*

In both groups, the cross-sectional area of the whole ACL increased relative to the control side (Table 2), but we found no significant difference between the groups.

**Table 2. Cross-sectional area ($mm^2$) of the ACL, mean (SD)**

| | Postoperative weeks | | |
|---|---|---|---|
| | 6 | 12 | 24 |
| Group F | | | |
| Experimental | 7.6 (1.4) | 7.4 (1.6) | 6.3 (0.8) |
| Control | 5.3 (0.6) | 5.0 (0.6) | 5.2 (0.2) |
| Exp./Control (%) | 146 | 141 | 121 |
| Group FM | | | |
| Experimental | 6.5 (0.6) | 7.7 (1.8) | 6.0 (1.2) |
| Control | 5.3 (0.8) | 5.2 (1.2) | 4.9 (0.5) |
| Exp./Control (%) | 127 | 149 | 119 |

### *Mechanical testing*

*1. Failure modes of the FATCs*. 3 failure modes were noted during the course of the study: rupture of the FATCs in the ligament substance, a tibial avulsion fracture, or a combination of both. The combined type occurred most often in both groups and in the control.

*2. Mechanical properties*. To determine the tensile strength, the specimens that failed because of a tibial avulsion fracture were excluded. A tangent modulus was determined in all specimens.

Postoperatively, we found grater reductions in the mean tensile strength and mean tangent modulus in the experimental sides than in the control sides in both groups (Tables 3 and 4). As compared to the ratio of the experimental to the control value, the tensile strength and tangent modulus in group FM were significantly lower than that in group F ($p = 0.004$, $p = 0.02$, ANOVA). At 24 weeks, both the tensile strength and tangent modulus, as compared to the ratio of the experimental to the control value, were lower in group FM than that in group F ($p = 0.004$, $p = 0.004$, Mann Whitney). In group F, the ratio of the tangent modulus had increased significantly at 24 weeks, as compared to that at 6 weeks ($p = 0.004$, Mann Whitney).

## Discussion

We hypothesized that in a combined grade III ACL and MCL injury, insufficiency of the medial structure affected the remodeling of the ACL graft.

**Table 3. Tensile strength (MPa) of the ACL, mean (SD)**

| | Postoperative weeks | | |
|---|---|---|---|
| | 6 | 12 | 24 |
| Group F | n = 4 | n = 5 | n = 4 |
| Experimental | 29 (9.5) | 35 (8.1) | 34 (2.6) |
| Control | 39 (12) | 46 (7.5) | 38 (5.8) |
| Exp./Control (%) | 75 | 76 | 93 |
| Group FM | n = 4 | n = 4 | n = 4 |
| Experimental | 29 (11) | 28 (1.6) | 31 (10) |
| Control | 49 (12) | 46 (7.8) | 45 (8.8) |
| Exp./Control (%) | 59 | 59 | 61 |

**Table 4. Tangent modulus (MPa) of the ACL, mean (SD)**

| | Postoperative weeks | | |
|---|---|---|---|
| | 6 | 12 | 24 |
| Group F | n = 5 | n = 5 | n = 5 |
| Experimental | 227 (85) | 220 (42) | 252 (91) |
| Control | 376 (83) | 310 (60) | 283 (70) |
| Exp./Control (%) | 60 | 71 | 89 |
| Group FM | n = 5 | n = 5 | n = 5 |
| Experimental | 169 (54) | 276 (85) | 224 (105) |
| Control | 312 (72) | 450 (139) | 332 (141) |
| Exp./Control (%) | 55 | 59 | 61 |

In our model, valgus instability persisted for 24 weeks, as found clinically in chronic cases.

In the present study, the mechanical properties of the in situ frozen ACLs combined with medial structure insufficiency were lower than those of the ACLs in normal medial structure.

Recently, several authors have reported in situ freeze-thaw treatment for the ACL (Jackson et al. 1991, Katsuragi et al. 2000). The model using this technique can simulate that of an ideally placed autogenous ACL graft. Therefore, we used the same model to exclude the effect of surgical technique on ACL reconstruction.

Earlier experimental studies have shown that the primary hindrance to valgus instability is the medial collateral ligament (Warren et al. 1974, Grood et al. 1981, Hughston 1994). When the MCL and medial structures are cut, this restraint is offset by the ACL (Grood et al. 1981, Inoue et al, 1987, Shapiro et al. 1991). Shapiro showed in the human cadaver that a section of the medial collateral ligament increased the force generated in the ACL when valgus moment was applied. In our study, valgus instability was significantly greater in group FM than in group F, and persisted for 24 weeks in group FM. It seems likely that the frozen ACL served as a restraint to valgus stress in this model.

Transplanted autogenous grafts undergo ischemic necrosis, revascularization, proliferation and remodeling (Arnoczky et al. 1982, Amiel et al. 1986a,b). The factors that affect remodeling in autogenous reconstruction include the graft material, methods of graft fixation, initial graft tension, graft position, rehabilitation after operation and concomitant ligamentous or meniscal injuries. If one excludes the graft material, these factors are related to the mechanical stress on the graft. A graft with good properties must have suitable tension. Because the ACL in group FM was a primary hindrance to valgus instability, the ACL suffered excessive valgus stress for 24 weeks after operation. The remodeling process was probably disrupted in group FM, which delayed the recovery of mechanical properties in the frozen ACL.

Our findings suggest that in a combined grade III ACL and MCL injury, surgical treatment, for example, repair or reconstruction of the MCL, is necessary, especially in chronic cases. In acute cases in which ACL reconstruction is performed, spontaneous healing of the MCL can be expected, along with a gradual reduction in valgus instability, but, in chronic cases, healing of the MCL can not be expected.

Our study had several limitations. It is uncertain whether or not the model we used is applicable to clinical grade III injury. However, by using rabbits we were able to evaluate many samples, and found that in both groups mechanical properties of the ACL tended to change.

No competing interests declared.

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